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(54) Title: MELANIN CONCENTRATING HORMONE RECEPTOR CHIMERIC AND FUSION PROTEINS

(57) Abstract: The present invention features melanin concentrating hormone receptor (MCH-R) chimeric and fusion proteins. MCH-R chimeric proteins comprise an MCH-R polypeptide region made up of at least two or more polypeptide regions characteristic of MCH-R found in different species. MCH-R fusion proteins comprise an MCH-R polypeptide region and a fluorescent protein region.

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TITLE OF THE INVENTION

MELANIN CONCENTRATING HORMONE RECEPTOR CHIMERIC AND
FUSION PROTEINS

5 CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims priority to provisional application U.S. Serial No. 60/189,698, filed March 15, 2000, hereby incorporated by reference herein.

BACKGROUND OF THE INVENTION

10 The references cited herein are not admitted to be prior art to the claimed invention.

Neuropeptides present in the hypothalamus play a major role in mediating the control of body weight. (Flier *et al.*, 1998. *Cell*, 92, 437-440.) Melanin-concentrating hormone (MCH) is a cyclic 19-amino acid neuropeptide synthesized as
15 part of a larger pre-prohormone precursor in the hypothalamus which also encodes neuropeptides NEI and NGE. (Nahon *et al.*, 1990. *Mol. Endocrinol.* 4, 632-637.) MCH was first identified in salmon pituitary, and in fish MCH affects melanin aggregation thus affecting skin pigmentation. In trout and in eels MCH has also been shown to be involved in stress induced or CRF-stimulated ACTH release. (Kawauchi
20 *et al.*, 1983. *Nature* 305, 321-323.)

In humans two genes encoding MCH have been identified that are expressed in the brain. (Breton *et al.*, 1993. *Mol. Brain Res.* 18, 297-310.) In mammals MCH has been localized primarily to neuronal cell bodies of the hypothalamus which are implicated in the control of food intake, including perikarya
25 of the lateral hypothalamus and zona inertia. (Knigge *et al.*, 1996. *Peptides* 17, 1063-1073.)

Pharmacological and genetic evidence suggest that the primary mode of MCH action is to promote feeding (orexigenic). MCH mRNA is up regulated in fasted mice and rats and in the *ob/ob* mouse. (Qu *et al.*, 1996. *Nature* 380, 243-247.)
30 Injection of MCH centrally (ICV) stimulates food intake and MCH antagonizes the hypophagic effects seen with α -melanocyte stimulating hormone (α MSH). (Qu *et al.*, 1996. *Nature* 380, 243-247.) MCH-deficient mice are lean, hypophagic, and have increased metabolic rate. (Shimada *et al.*, 1998. *Nature* 396, 670-673.)

MCH action is not limited to modulation of food intake as effects on
35 the hypothalamic-pituitary-axis have been reported. (Nahon 1994. *Critical Rev. in*

Neurobiol. 8, 221-262.) MCH may be involved in the body response to stress as MCH can modulate the stress-induced release of CRF from the hypothalamus and ACTH from the pituitary. In addition, MCH neuronal systems may be involved in reproductive or maternal function.

5 Several references describe a receptor that is indicated to bind MCH. (Chambers *et al.*, 1999. *Nature* 400, 261-265; Saito *et al.*, 1999. *Nature* 400, 265-269; Bächner *et al.*, 1999. *FEBS Letters* 457:522-524; Shimomura *et al.*, 1999. *Biochemical and Biophysical Research Communications* 261, 622-626; and Lembo *et al.*, 1999. *Nat. Cell Biol.* 1, 267-271.)

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SUMMARY OF THE INVENTION

The present invention features melanin concentrating hormone receptor (MCH-R) chimeric and fusion proteins. MCH-R chimeric proteins comprise an MCH-R polypeptide region made up of at least two or more polypeptide regions
15 characteristic of MCH-R found in different species. MCH-R fusion proteins comprise an MCH-R polypeptide region and a fluorescent protein region.

An MCH-R polypeptide region provides a functional G-protein coupled receptor region able to bind MCH and transduce an intracellular signal. Examples of MCH-R polypeptide regions include naturally occurring MCH-R,
20 chimeric MCH-R containing two or more regions from naturally occurring MCH-R, and functional derivatives thereof.

Reference to the terms "characteristic" and "derivatives thereof" describe a relationship to a reference sequence. In both cases, there is at least about 75% sequence similarity to the reference sequence.

25 Thus, a first aspect of the present invention describes a fusion protein comprising (a) an MCH-R polypeptide region and (b) a fluorescent polypeptide region. The fluorescent polypeptide region is joined directly, or through a polypeptide linker, to the carboxy side of the MCH-R polypeptide region.

Another aspect of the present invention describes an MCH-R chimeric
30 protein. The protein comprises: (a) an MCH-R binding region characteristic of a human MCH-R, (b) a transmembrane domain characteristic of a non-human MCH-R, and (c) an intracellular domain characteristic of a non-human MCH-R.

Another aspect of the present invention describes a nucleic acid encoding for an MCH-R fusion protein or an MCH-R chimeric protein described
35 herein. Such nucleic acid comprises either a contiguous nucleotide sequence that

codes for the protein or a sequence that is processed by a host cell to produce a contiguous nucleotide sequence encoding for the protein. Processing of a nucleic acid sequence to produce a contiguous nucleotide sequence encoding for a protein can occur by the splicing together of exons resulting in intron removal.

5 Another aspect of the present invention describes an expression vector comprising a nucleic acid encoding for an MCH-R fusion protein or an MCH-R chimeric protein described herein.

10 Another aspect of the present invention describes a recombinant cell comprising nucleic acid encoding for an MCH-R fusion protein or an MCH-R chimeric protein described herein. The nucleic acid may be part of the host genome or may exist independently of the host genome.

Another aspect of the present invention describes a non-human transgenic animal comprising nucleic acid encoding for an MCH-R fusion protein or an MCH-R chimeric protein described herein.

15 Another aspect of the present invention describes a method for assaying for MCH-R active compounds by measuring the effect of a test preparation on one or more MCH-R activities. The method is performed using either an MCH-R fusion protein or an MCH-R chimeric protein described herein.

20 Other features and advantages of the present invention are apparent from the additional descriptions provided herein including the different examples. The provided examples illustrate different components and methodology useful in practicing the present invention. The examples do not limit the claimed invention. Based on the present disclosure the skilled artisan can identify and employ other components and methodology useful for practicing the present invention.

25

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates aequorin assay results comparing a mouse MCH-R fusion with a human wild type MCH-R and a CMV-EGFP control.

30 Figure 2 illustrates a cAMP flashplate assay of CHO cell clones stably expressing mMCH-1R-EGFP. Cells from individual clones were dissociated in enzyme free media and stimulated for 15 minutes at 37°C with human MCH at the indicated concentrations in the presence of 10 µM forskolin. Cells were then lysed and assayed for bound [¹²⁵I]cAMP. Mouse MCH-1R-EGFP clones exhibited EC50 values (0.1111, 0.1255, 0.1291, or 0.2304 nM) indistinguishable from that of a CHO
35 cell clone expressing the wild-type human short isoform of MCH-1R (0.1282 nM).

Figure 3 illustrates a cAMP flashplate assay of CHO cell clones stably expressing human short/mouse species chimeric MCH-1R-EGFP. Cells from individual clones were dissociated in enzyme free media and stimulated for 15 minutes at 37°C with human MCH at the indicated concentrations in the presence of 10 µM forskolin. Cells were then lysed and assayed for bound [¹²⁵I]cAMP. Human short/mouse species chimeric MCH-1R-EGFP clones exhibited EC50 values (0.0366, 0.0462, 0.2117, or 0.2499 nM) indistinguishable from that of a CHO cell clone expressing the wild-type human short isoform of MCH-1R (0.1137 nM).

10 DETAILED DESCRIPTION OF THE INVENTION

The present invention features MCH-R chimeric and fusion proteins. Such proteins have a variety of different uses including being used as a research tool to study MCH-R function and dynamics, and being used to screen for MCH-R agonists and antagonists.

15 The MCH-R provides a target to achieve different beneficial effects in a patient. Preferably, MCH-R activity is modulated to achieve one or more of the following: weight loss, weight gain, treat cancer (*e.g.*, colon or breast), reduce pain, treat diabetes, reduce stress, or treat sexual dysfunction.

Modulation of MCH-R activity can be achieved by evoking a response at the MCH receptor or by altering a response evoked by an MCH receptor agonist or antagonist. Compounds modulating MCH-R receptor activity include agonists, antagonists, and allosteric modulators. Generally, MCH-R antagonists and allosteric modulators negatively affecting activity will be used to achieve weight loss, treat cancer (*e.g.*, colon or breast), reduce pain, reduce stress, or treat sexual dysfunction; and MCH-R agonists and allosteric modulators positively affecting activity will be used to produce a weight gain.

20 Preferably, MCH-R activity is modulated to achieve a weight loss or to treat diabetes in a patient. Diabetes mellitus can be treated by modulating MCH-R activity to achieve, for example, one or both of the following: enhancing glucose tolerance or decreasing insulin resistance.

30 Excessive body weight is a contributing factor to different diseases, including hypertension, diabetes, dyslipidemias, cardiovascular disease, gall stones, osteoarthritis, and certain forms of cancers. Bringing about a weight loss can be used, for example, to reduce the likelihood of such diseases and as part of a treatment for such diseases. Weight reduction can be achieved by modulating MCH-R activity to

obtain, for example, one or more of the following effects: reducing appetite, increasing metabolic rate, reducing fat intake, or reducing carbohydrate craving.

Increasing body weight is particularly useful for a patient having a disease or disorder, or under going a treatment, accompanied by weight loss.

- 5 Examples of diseases or disorders accompanied by weight loss include anorexia, AIDS, wasting, cachexia, and frail elderly. Examples of treatments accompanied by weight loss include chemotherapy and radiation therapy.

MCH-R Chimeric Proteins

- 10 MCH-R chimeric proteins contain an MCH-R polypeptide region made up by at least two or more polypeptide regions characteristic of MCH-R found in different species. The different polypeptide regions that are present provide for an N-terminal extracellular domain; a transmembrane domain made up of transmembrane regions, extracellular loop regions, and intracellular loop regions; and an intracellular
15 carboxy terminus domain. Examples of MCH-R amino acid sequences include the following: SEQ. ID. NO. 1 (human MCH1R long form), SEQ. ID. NO. 2 (human MCH1R short form), and SEQ. ID. NO. 3 (mouse MCH1R).

- Preferably, the MCH-R chimeric protein comprises an MCH-R binding region characteristic of a human MCH-R along with transmembrane and intracellular
20 domains characteristic of a non-human MCH-R. There are substantial amino acid differences between the N-terminus of the MCH-R present in humans and that present in other species such as mice. Such differences could result in, for example, the mouse MCH-R having different intrinsic properties and responsiveness to agonists and/or antagonists than the human MCH-R. The presence of a human MCH-R
25 binding region provides for a "humanized" MCH-R chimeric receptor.

- The transmembrane and intracellular domains characteristic of a non-human MCH-R can be used in conjunction with a non-human host to provide a more naturally occurring environment for these regions. For example, an MCH-R chimeric having mouse transmembrane and intracellular domains are preferably used in murine
30 cells lines or in transgenic mice.

MCH-R chimeric proteins may contain regions other than extracellular, transmembrane, and intracellular domains that do not substantially decrease the activity of the protein. Preferably, additional regions do not cause a decrease of more than about 25% of MCH-R activity as measured using one or more of the assays

described in the examples provided below. Examples of additional regions that may be present include fluorescent protein regions and linker regions.

In an embodiment of the present invention, the MCH-R chimeric protein comprises: (a) an MCH binding region characteristic of a first species and (b) a transmembrane and intracellular domain region characteristic of a second species joined directly, or through a linker, to the carboxy side of the MCH binding region. Preferably, the protein comprises, consists, or consists essentially of an MCH-R polypeptide having a sequence similarity of at least about 75%, at least 85%, or at least 95% with either SEQ. ID. NO. 4 (human short form/mouse species chimeric MCH1R) or SEQ. ID. NO. 5 (human long form/mouse species chimeric). Even more preferably, the protein comprises, consists essentially of, or consists of, SEQ. ID. NO. 4 or SEQ. ID. NO. 5.

Sequence similarity for polypeptides can be determined by BLAST. (Altschul *et al.*, 1997. *Nucleic Acids Res.* 25, 3389-3402, hereby incorporated by reference herein.) In an embodiment of the present invention, sequence similarity is determined using tBLASTn search program with the following parameters: MATRIX:BLOSUM62, PER RESIDUE GAP COST: 11, and Lambda ratio: 1.

Differences in naturally occurring amino acids are due to different R groups. An R group effects different properties of the amino acid such as physical size, charge, and hydrophobicity. Amino acids can be divided into different groups as follows: neutral and hydrophobic (alanine, valine, leucine, isoleucine, proline, tryptophan, phenylalanine, and methionine); neutral and polar (glycine, serine, threonine, tyrosine, cysteine, asparagine, and glutamine); basic (lysine, arginine, and histidine); and acidic (aspartic acid and glutamic acid).

Generally, in substituting different amino acids it is preferable to exchange amino acids having similar properties. Substituting different amino acids within a particular group, such as substituting valine for leucine, arginine for lysine, and asparagine for glutamine are good candidates for not causing a change in polypeptide functioning.

Changes outside of different amino acids groups can also be made. Preferably, such changes are made taking into account the position of the amino acid to be substituted in the polypeptide. For example, arginine can substitute more freely for nonpolar amino acids in the interior of a polypeptide than glutamate because of its long aliphatic side chain. (See, Ausubel, *Current Protocols in Molecular Biology*, John Wiley, 1987-1998, Supplement 33 Appendix 1C.)

MCH-R Fusion Proteins

5 MCH-R fusion proteins contain an MCH-R polypeptide region and a fluorescent protein region either directly joined together or joined together through a linker. These regions provide MCH-R activity and a marker for evaluating MCH-R dynamics.

An MCH-R polypeptide region provides functional MCH-R activity and includes naturally occurring MCH-R, chimeric MCH-R, and derivatives thereof. Preferred derivatives thereof have a sequence similarity of at least about 75%, at least
10 about 85%, or at least about 95% to a naturally occurring MCH-R or a chimeric MCH-R described herein.

A fluorescent protein region contains a chromophore that fluoresces. Preferably, the fluorescent protein region is the green fluorescent protein of the jellyfish *Aequorea victoria* or a derivative thereof. Preferred derivatives have a
15 sequence similarity of at least about 75%, at least about 85%, or at least about 95% to the *Aequorea victoria* green fluorescent protein (GFP). The *Aequorea victoria* green fluorescent protein and examples of derivatives thereof are described by Cormack *et al.*, 1996. *Gene* 17, 33-38; Yang *et al.*, 1996. *Nucleic Acids Research* 24, 4592-4593; Tsien *et al.*, U.S. Patent No. 5,625,048; Tsien *et al.*, U.S. Patent No. 5,777,079; and
20 Cormack *et al.*, U.S. Patent No. 5,804,387 (each of which are hereby incorporated by reference herein).

In different embodiments the MCH-R polypeptide region comprises, consists essentially of, or consists of, a sequence selected from the group consisting of: SEQ. ID. NO. 1, SEQ. ID. NO. 2, SEQ. ID. NO. 3, SEQ. ID. NO. 4, and SEQ. ID.
25 NO. 5; and the fluorescent polypeptide region comprises, consists essentially of, or consists of, an amino acid sequence selected from the group consisting of SEQ. ID. NO. 6 (GFP), SEQ. ID. NO. 7 (EGFP), SEQ. ID. NO. 8 (Emerald), SEQ. ID. NO. 9 (Topaz), and SEQ. ID. NO. 10 (W1b). EGFP, Emerald, Topaz, and W1b are derivatives of GFP.

30 The optionally present linker is a polypeptide region that is preferably from 1 to about 100 amino acids in length. In different embodiments the linker is up to 75, 50 or 25 amino acids in length.

Preferably, the MCH-R fusion protein comprises, consists essentially of, or consists of, the MCH-R polypeptide region and the fluorescent polypeptide
35 region. More preferably, the protein comprises, consists essentially of, or consists of,

an amino acid sequence selected from the group consisting of: SEQ. ID. NO. 11 (mouse MCH1R-linker-EGFP), SEQ. ID. NO. 12 (mouse MCH1R/EGFP direct fusion), SEQ. ID. NO. 13 (human short form/mouse species chimeric MCH1R-linker-EGFP), or SEQ. ID. NO. 14 (human long form/mouse species chimeric MCH1R-linker-EGFP).

MCH-R Chimeric and Fusion Proteins Nucleic Acid and Expression

MCH-R chimeric and fusion proteins can be produced using techniques well known in the art. Preferably, such proteins are produced by recombinant expression inside a host cell by way of an expression vector or by way of nucleic acid integrated into the host genome. Examples of nucleic acid sequences encoding for MCH-R polypeptide regions, fluorescent protein regions, MCH-R chimeric proteins, and MCH-R fusion proteins are provided for by SEQ. ID. NOs. 15-29 (see Example 1, *infra*).

Starting with a particular amino acid sequence and the known degeneracy of the genetic code, a large number of different encoding nucleic acid sequences can be obtained. The degeneracy of the genetic code arises because almost all amino acids are encoded for by different combinations of nucleotide triplets or codons. The translation of a particular codon into a particular amino acid is well known in the art (see, e.g., Lewin *GENES IV*, p. 119, Oxford University Press, 1990).

Amino acids are encoded for by codons as follows:

- A=Ala=Alanine: codons GCA, GCC, GCG, GCU
- C=Cys=Cysteine: codons UGC, UGU
- D=Asp=Aspartic acid: codons GAC, GAU
- E=Glu=Glutamic acid: codons GAA, GAG
- F=Phe=Phenylalanine: codons UUC, UUU
- G=Gly=Glycine: codons GGA, GGC, GGG, GGU
- H=His=Histidine: codons CAC, CAU
- I=Ile=Isoleucine: codons AUA, AUC, AUU
- K=Lys=Lysine: codons AAA, AAG
- L=Leu=Leucine: codons UUA, UUG, CUA, CUC, CUG, CUU
- M=Met=Methionine: codon AUG
- N=Asn=Asparagine: codons AAC, AAU
- P=Pro=Proline: codons CCA, CCC, CCG, CCU
- Q=Gln=Glutamine: codons CAA, CAG

R=Arg=Arginine: codons AGA, AGG, CGA, CGC, CGG, CGU

S=Ser=Serine: codons AGC, AGU, UCA, UCC, UCG, UCU

T=Thr=Threonine: codons ACA, ACC, ACG, ACU

V=Val=Valine: codons GUA, GUC, GUG, GUU

5 W=Trp=Tryptophan: codon UGG

Y=Tyr=Tyrosine: codons UAC, UAU

Examples of techniques for introducing nucleic acid into a cell and expressing the nucleic acid to produce protein are provided in references such as Ausubel, *Current Protocols in Molecular Biology*, John Wiley, 1987-1998, and
10 Sambrook, *et al.*, in *Molecular Cloning, A Laboratory Manual*, 2nd Edition, Cold Spring Harbor Laboratory Press, 1989.

An expression vector contains recombinant nucleic acid encoding for a polypeptide along with regulatory elements for proper transcription and processing. The recombinant nucleic acid contains two or more nucleic acid regions not naturally
15 associated with each other. Exogenous regulatory elements such as an exogenous promoter can be useful for expressing recombinant nucleic acid in a particular host. Examples of expression vectors are cloning vectors, modified cloning vectors, specifically designed plasmids, and viruses.

Generally, the regulatory elements that are present in an expression
20 vector include a transcriptional promoter, a ribosome binding site, a terminator, and an optionally present operator. Another preferred element is a polyadenylation signal providing for processing in eukaryotic cells. Preferably, an expression vector also contains an origin of replication for autonomous replication in a host cell, a selectable marker, a limited number of useful restriction enzyme sites, and a potential for high
25 copy number.

Expression vectors providing suitable levels of polypeptide expression in different hosts are well known in the art. Mammalian expression vectors well known in the art include pcDNA3 (Invitrogen), pMC1neo (Stratagene), pXT1 (Stratagene), pSG5 (Stratagene), EBO-pSV2-neo (ATCC 37593), pBPV-1(8-2)
30 (ATCC 37110), pdBPV-MMTneo(342-12) (ATCC 37224), pRSVgpt (ATCC 37199), pRSVneo (ATCC 37198), pSV2-dhfr (ATCC 37146), pUCTag (ATCC 37460), pCI-neo (Promega) and .lambda.ZD35 (ATCC 37565). Bacterial expression vectors well known in the art include pET11a (Novagen), lambda gt11 (Invitrogen), pcDNAII (Invitrogen), and pKK223-3 (Pharmacia). Fungal cell expression vectors well known

in the art include pYES2 (Invitrogen) and Pichia expression vector (Invitrogen).
Insect cell expression vectors well known in the art include Blue Bac III (Invitrogen).

Recombinant host cells may be prokaryotic or eukaryotic. Examples
of recombinant host cells include the following: bacteria such as *E. coli*; fungal cells
5 such as yeast; mammalian cells such as human, bovine, porcine, monkey, hamster,
and rodent; and insect cells such as *Drosophila* and silkworm derived cell lines.
Commercially available mammalian cell lines include L cells L-M(TK.sup.-) (ATCC
CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL
86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651),
10 CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658),
HeLa (ATCC CCL 2), C1271 (ATCC CRL 1616), BS-C-1 (ATCC CCL 26) and
MRC-5 (ATCC CCL 171).

To enhance expression in a particular host it may be useful to modify
the sequence to take into account codon usage of the host. Codon usage of different
15 organisms are well known in the art. (See, Ausubel, *Current Protocols in Molecular
Biology*, John Wiley, 1987-1998, Supplement 33 Appendix 1C.)

Expression vectors may be introduced into host cells using standard
techniques. Examples of such techniques include transformation, transfection,
lipofection, protoplast fusion, and electroporation.

20 Nucleic acid encoding for a polypeptide can be expressed in a cell
without the use of an expression vector employing, for example, synthetic mRNA or
native mRNA. Additionally, mRNA can be translated in various cell-free systems
such as wheat germ extracts and reticulocyte extracts, as well as in cell based systems,
such as frog oocytes. Introduction of mRNA into cell based systems can be achieved,
25 for example, by microinjection.

Techniques for producing transgenic animals are well known in the art.
Examples of such techniques are provided for by Teratocarcinomas and embryonic
stem cells: a practical approach. Ed. By E. J. Robertson, IRL Press Limited, Oxford,
England (1987); and Gene Targeting: a practical approach. Ed. By A. L. Joyner,
30 Oxford University Press Inc. New York, NY (1993).

G-Protein Coupled Receptor Assays

MCH-R is G-protein coupled receptor. Techniques for measuring
different G-protein activities, such as Gi/o, Gs, and Gq are well known in the art.
35 MCH-R activity is preferably assayed for by measuring either Gi/o or Gq.

Gi/o and Gs activity can be measured using techniques such as a melonaphore assay, measuring cAMP production, measuring inhibition of cAMP accumulation, and measuring binding of ^{35}S -GTP. cAMP can be measured using different techniques such as radioimmunoassay and indirectly by cAMP responsive gene reporter proteins.

Gq activity can be measured using techniques such as those measuring intracellular Ca^{2+} . Examples of techniques well known in the art that can be employed to measure Ca^{2+} include the use of dyes such as Fura-2 and the use of Ca^{2+} -bioluminescent sensitive reporter proteins such as aequorin. An example of a cell line employing aequorin to measure G-protein activity is HEK293/aeq17. (Button *et al.*, 1993. *Cell Calcium* 14, 663-671, and Feighner *et al.*, 1999. *Science* 284, 2184-2188, both of which are hereby incorporated by reference herein.)

Functional assays can be performed using individual compounds or preparations containing different compounds. A preparation containing different compounds where one or more compounds affect MCH-R chimeric or fusion protein activity can be divided into smaller groups of compounds to identify the compound(s) affecting MCH-R chimeric or fusion protein activity. In an embodiment of the present invention a test preparation containing at least 10 compounds is used in a functional assay.

Functional assays can be performed using recombinantly produced MCH-R chimeric or fusion protein present in different environments. Such environments include, for example, cell extracts and purified cell extracts containing the MCH-R chimeric or fusion protein expressed from recombinant nucleic acid and an appropriate membrane for the polypeptide; and the use of a purified MCH-R chimeric or fusion protein produced by recombinant means that is introduced into a different environment suitable for measuring G-protein activity.

Fluorescent Protein Assays

Fluorescent protein joined to an MCH receptor can be employed to study different aspects of receptor dynamics including receptor sequestration, receptor desensitization, and receptor localization. The fluorescent protein can be used in *in vitro* or *in vivo* systems.

In vitro applications of fluorescent proteins can be performed using techniques well known in the art. Examples of such techniques are provided by Barak *et al.*, 1997. *Mol Pharm.* 5, 177-184; Tarasova *et al.*, 1997. *J. Biol. Chem.* 272,

- 14817-14824; Lin *et al.*, 1998. *Mol. Cell. Endo.* 146, 27-37; Tarasova *et al.*, 1998. *J. Biol. Chem.* 273, 15883-15886; Kallal *et al.*, 1998. *J. Biol. Chem.* 273, 322-328; Groake *et al.*, 1999. *J. Biol. Chem.* 274, 23263-23269; Doherty *et al.*, 1999. *Biochem. J.* 341, 415-422; Brock *et al.*, 1999. *Proc. Natl. Acad. Sci. USA* 96, 10123-10128; Cornea *et al.*, 1999. *Endocrinology* 140, 4272-4280; and Lembo *et al.*, 1999. *Nat. Cell Biol.* 1, 267-271 (these references are not admitted to be prior art to the claimed invention).

- In vivo* applications of fluorescent proteins can be performed using techniques well known in the art. Examples of such techniques are provided by Mombaerts *et al.*, 1996. *Cell* 87, 675-686; Rodriguez *et al.*, 1999. *Cell* 97, 199-208; Spergel *et al.*, 1999. *J. Neurosci.* 1, 2037-2050; and Zuo *et al.*, 1999. *Proc. Natl. Acad. Sci. USA* 96, 14100-14105 (these references are not admitted to be prior art to the claimed invention).

EXAMPLES

- Examples are provided below to further illustrate different features and advantages of the present invention. The examples also illustrate useful methodology for practicing the invention. These examples do not limit the claimed invention.

Example 1:

- Amino acid and nucleic acid sequence information for SEQ. ID. NOs. 1-29 are provided below. SEQ. ID. NOs. 1-29 include examples of polypeptide and encoding nucleic acid sequences for MCH-R polypeptide regions, fluorescent polypeptide regions, fusion proteins and chimeric proteins. In some cases the encoding nucleic acid is shown with additional nucleic acid upstream or downstream from an open reading frame.

SEQ. ID. NO. 1: Human long form MCH1R

- MSVGAMKKGVGRAVGLGGSGCQATEEDPLPNCGACAPGQGGRWRWLPQP
AWVEGSSARLWEQATGTGWMDLEASLLPTGPNASNTSDGPDNLTSAGSPPR
TGSISYINIIMPSVFGTICLLGIIGNSTVIFAVVKKSKLHWCNNVPDIFIINLSVVD
LLFLLGMPFMIHQLMGNGVWHFGETMCTLITAMDANSQFTSTYILTAMAI DR
YLATVHPISSTKFRKPSVATLVICLLWALSFISITPVWLYARLIPFGGAVGCGI
RLPNPDTDLYWFTLYQFFLAFALPFVVITAAYVRILQRMTSSVAPASQRSIRLR

TKRVTRTAIAICLVFFVCWAPYYVLQLTQLSISRPTLTFVYLYNAAISLGYANS
CLNPFVYIVLCETFRKRLVLSVKPAAQGQLRAVSNAQTADEERTESKGT

SEQ. ID. NO. 2: Human short form MCH1R

5 MDLEASLLPTGPNASNTSDGPDNLTSAGSPPRTGSGSYINIIMPSVFGTICLLGIIG
NSTVIFAVVKKSKLHWCNNVPDIFIINLSVVDLLFLLGMPFMIHQLMGNGVWH
FGETMCTLITAMDANSQFTSTYILTAMADRYLATVHPISSTKFRKPSVATLVI
CLLWALSFSITPVWLYARLIPFPGGAVGCGIRLPNPDTDLWFTLYQFFLAFA
LPPFVVITAAYVRILQRMSTSSVAPASQRSIRLRTKRVTRTAIAICLVFFVCWAPY
10 YVLQLTQLSISRPTLTFVYLYNAAISLGYANSCLNPFVYIVLCETFRKRLVLSV
KPAAQGQLRAVSNAQTADEERTESKGT

SEQ. ID. NO. 3: Mouse MCH1R

MDLQASLLSTGPNASNISDGQDNFTLAGPPPRTRSVSYINIIMPSVFGTICLLGI
15 VGNSTVIFAVVKKSKLHWCNNVPDIFIINLSVVDLLFLLGMPFMIHQLMGNGV
WHFGETMCTLITAMDANSQFTSTYILTAMADRYLATVHPISSTKFRKPSMAT
LVICLLWALSFSITPVWLYARLIPFPGGAVGCGIRLPNPDTDLWFTLYQFFLA
FALPPFVVITAAYVKILQRMSTSSVAPASQRSIRLRTKRVTRTAIAICLVFFVCWA
PYVVLQLTQLSISRPTLTFVYLYNAAISLGYANSCLNPFVYIVLCETFRKRLVLS
20 VKPAAQGQLRTVSNAQTADEERTESKGT

SEQ. ID. NO. 4: Human short form/mouse species chimeric MCH1R

MDLEASLLPTGPNASNTSDGPDNLTSAGSPPRTGSGSYINIIMPSVFGTICLLGIIG
NSTVIFAVVKKSKLHWCNNVPDIFIINLSVVDLLFLLGMPFMIHQLMGNGVWH
25 FGETMCTLITAMDANSQFTSTYILTAMADRYLATVHPISSTKFRKPSMATLVI
CLLWALSFSITPVWLYARLIPFPGGAVGCGIRLPNPDTDLWFTLYQFFLAFA
LPPFVVITAAYVKILQRMSTSSVAPASQRSIRLRTKRVTRTAIAICLVFFVCWAPY
YVLQLTQLSISRPTLTFVYLYNAAISLGYANSCLNPFVYIVLCETFRKRLVLSV
KPAAQGQLRTVSNAQTADEERTESKGT

30

SEQ. ID. NO. 5: Human long form/mouse species chimeric MCH1R

MSVGAMKKGVGRAVGLGGSGCQATEEDPLPNCGACAPGQGGRWRPQP
AWVEGSSARLWEQATGTGWMDEASLLPTGPNASNTSDGPDNLTSAGSPPR
TGSISYINIIMPSVFGTICLLGIIGNSTVIFAVVKKSKLHWCNNVPDIFIINLSVVD
35 LLFLLGMPFMIHQLMGNGVWHFGETMCTLITAMDANSQFTSTYILTAMADR

YLATVHPISSTKFRKPSMATLVICLLWALSFISITPVWLYARLIPFPGGAVGCGI
 RLPNPD TDLYWFTLYQFFLAFALPFVVITAAAYVKILQRM TSSVAPASQRSIRLR
 TKRVTRTAIAICLVFFVCWAPYYVLQLTQLSISRPTLTFVYLYNAAISLGYANS
 CLNPFVYIVLCETFRKRLVLSVKPAAQGGQLRTVSNAQTAD EERTESKGT

5

SEQ. ID. NO. 6: GFP

MSKGEELFTGVVPILVELDGDVNGHKFSVS GEGEGDATY GKLTLKFICTTGKL
 PVPWP TLVTTFSYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGN
 YKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYN SHNVYIMADKQ
 10 KNGIKVNFKIRHNIEDGSVQLADHYQNTPIGDGPVLLPDNHYLSTQSALSKD
 PNEKRDH MVLLEFVTAAGITHGMDELYK

SEQ. ID. NO. 7: EGFP

MVSKGEELFTGVVPILVELDGDVNGHKFSVS GEGEGDATY GKLTLKFICTTGK
 15 LPVPWP TLVTTLT YGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDG
 NYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYN SHNVYIMADK
 QKNGIKVNFKIRHNIEDGSVQLADHYQNTPIGDGPVLLPDNHYLSTQSALSK
 DPNEKRDH MVLLEFVTAAGITLGMDELYK

20 SEQ. ID. NO. 8: Emerald

Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp
 Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr
 Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr
 Leu Val Thr Thr Leu Thr Tyr Gly Val Gln Cys Phe Ala Arg Tyr Pro Asp His Met Lys
 25 Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe
 Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr
 Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly
 His Lys Leu Glu Tyr Asn Tyr Asn Ser His Lys Val Tyr Ile Thr Ala Asp Lys Gln Lys
 Asn Gly Ile Lys Val Asn Phe Lys Thr Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu
 30 Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn
 His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met
 Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys

SEQ. ID. NO. 9: T paz

Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp
 Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr
 Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr
 5 Leu Val Thr Thr Phe Gly Tyr Gly Val Gln Cys Phe Ala Arg Tyr Pro Asp His Met Arg
 Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe
 Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr
 Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly
 His Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys
 10 Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu
 Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn
 His Tyr Leu Ser Tyr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met
 Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys

15 SEQ. ID. NO. 10: W1B

Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp
 Gly Asp Val Asn Gly His Arg Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr
 Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr
 Leu Val Thr Thr Leu Thr Trp Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
 20 Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe
 Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr
 Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly
 His Lys Leu Glu Tyr Asn Tyr Ile Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln Lys
 Asn Gly Ile Lys Ala His Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu
 25 Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn
 His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met
 Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys

SEQ. ID. NO. 11: Mouse MCH1R-linker-EGFP

30 MDLQASLLSTGPNASNISDGQDNFTLAGPPPRTRSVSYINIIMPSVFGTICLLGI
 VGNSTVIFAVVKKSKLHWCSNVPDIFIINLSVVDLLFLLGMPFMIHQLMGNGV
 WHFGETMCTLITAMDANSQFTSTYILTAMAIIDRYLATVHPISSTKFRKPSMAT
 LVICLLWALSFISITPVWLYARLIPFGGAVGCGIRLPNPD TDLYWFTLYQFFLA
 FALPFVVITAAYVKILQRMTSSVAPASQRSIRLRTKRVRTAIAICLVFFVCWA
 35 PYYVLQLTQLSISRPTLTFVYLYNAAISLGYANSCLNPFVYIVLCETFRKRLVLS

VKPAAQGQLRTVSNAQTADERTESKGTVDGTAGPGSIATMVSKGEELFTGV
 VPILVELDGDVNGHKFSVSGEGEDATYGKLTCLKFICTTGKLPVPWPTLVTTL
 TYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFE
 GDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIR
 5 HNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMLV
 LEFVTAAGITLGMDELYK

SEQ. ID. NO. 12: Mouse MCH1R/EGFP direct fusion

MDLQASLLSTGPNASNISDGQDNFTLAGPPRTRSVSYNIIMPSVFGTICLLGI
 10 VGNSTVIFAVVKKSKLHWCNNVPDIFIINLSVVDLLFLLGMPFMIHQLMGNGV
 WHFGETMCTLITAMDANSQFTSTYILTAMADRYLATVHPISSTKFRKPSMAT
 LVICLLWALSFSITPVWLYARLIPFPGGAVGCGIRLPNPDTDLWFTLYQFFLA
 FALPFVVITAAYVKILQRMTSSVAPASQRSIRLRTKRVTRTAIAICLVFFVCWA
 PYYVLQLTQLSISRPTLTFVYLYNAAISLGYANSCLNPFVYIVLCETFRKRLVLS
 15 VKPAAQGQLRTVSNAQTADERTESKGTMVSKGEELFTGVVPILVELDGDVN
 GHKFSVSGEGEDATYGKLTCLKFICTTGKLPVPWPTLVTTLTYYGVQCFSRYP
 HMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFE GDTLVNRIELKGI
 DFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLAD
 HYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMLVLEFVTAAGITLG
 20 MDELYK

SEQ. ID. NO. 13: Human short form/mouse species chimeric MCH1R-linker-EGFP

MDLEASLLPTGPNASNTSDGPDNLTSAGSPPRTGSIYNIIMPSVFGTICLLGI
 25 NSTVIFAVVKKSKLHWCNNVPDIFIINLSVVDLLFLLGMPFMIHQLMGNGVWH
 FGETMCTLITAMDANSQFTSTYILTAMADRYLATVHPISSTKFRKPSMATLVI
 CLLWALSFSITPVWLYARLIPFPGGAVGCGIRLPNPDTDLWFTLYQFFLAFA
 LPFVVITAAYVKILQRMTSSVAPASQRSIRLRTKRVTRTAIAICLVFFVCWAPY
 YVLQLTQLSISRPTLTFVYLYNAAISLGYANSCLNPFVYIVLCETFRKRLVLSV
 30 KPAAQGQLRTVSNAQTADERTESKGTVDGTAGPGSIATMVSKGEELFTGVV
 PILVELDGDVNGHKFSVSGEGEDATYGKLTCLKFICTTGKLPVPWPTLVTTLT
 YGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFE G
 DTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRH
 NIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMLVLL
 35 EFVTAAGITLGMDELYK

SEQ. ID. NO. 14: Human long form/mouse species chimeric MCH1R-linker-EGFP

MSVGAMKKGVGRAVGLGGGSGCQATEEDPLPNCGACAPGQGRRWRLPQP
5 A WVEGSSARLWEQATGTGWMDLEASLLPTGPNASNTSDGPDNLTSA GSPPR
TGSISYNIIMPSVFGTICLLGIIGNSTVIFAVVKKSKLHWCNNVPDIFIINLSVVD
LLFLLGMPFMIHQLMGNGVWHFGETMCTLITAMDANSQFTSTYILTAMADR
YLATVHPISSTKFRKPSMATLVICLLWALSFISITPVWLYARLIPFPGGAVGCGI
RLPNPD TDLYWFTLYQFFLAFALPFVVITAA YVKILQRMTSSVAPASQRSIRLR
10 TKRVTRTAIAICLVFFVCWAPYYVLQLTQLSISRPTLTFVYLYNAAISLGYANS
CLNPFVYIVLCETFRKRLVLSVKPAAQGQLRTVSNAQTADERTESKGTVDGT
AGPGSIATMVSKGEELFTGVVPILVELDGDVNGHKFSVS GEGEGDATY GKLT
LKFICTTGKLPVPWPTLVTTLT YGVQCFSRYPDHMKQHDFFKSAMP EGYVQE
RTIFFKDDGNYKTRA EVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSH
15 NVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHY
LSTQSALSKDPNEKRDH MVLLEFVTAAGITLGMD ELYK

SEQ. ID. NO. 15: Human long form MCH1R cDNA

ATGTCAGTGGGAGCCATGAAGAAGGGAGTGGGGAGGGCAGTTGGGCTTG
20 GAGGCGGCAGCGGCTGCCAGGCTACGGAGGAAGACCCCTTCCCAACTGC
GGGGCTTGCGCTCCGGGACAAGGTGGCAGGCGCTGGAGGCTGCCGCAGC
CTGCGTGGGTGGAGGGGAGCTCAGCTCGGTTGTGGGAGCAGGCGACCGG
CACTGGCTGGATGGACCTGGAAGCCTCGCTGCTGCCCCACTGGTCCCAACG
CCAGCAACACCTCTGATGGCCCCGATAACCTCACTTCGGCAGGATCACCT
25 CCTCGCACGGGGAGCATCTCCTACATCAACATCATCATGCCTTCGGTGTTT
GGCACCATCTGCCTCCTGGGCATCATCGGGAACCTCCACGGTCATCTTCGCG
GTCGTGAAGAAGTCCAAGCTGCACTGGTGCAACAACGTCCCCGACATCTT
CATCATCAACCTCTCGGTAGTAGATCTCCTCTTTCTCCTGGGCATGCCCTT
CATGATCCACCAGCTCATGGGCAATGGGGTGTGGCACTTTGGGGAGACCA
30 TGTGCACCCTCATCACGGCCATGGATGCCAATAGTCAGTTCACCAGCACC
TACATCCTGACCGCCATGGCCATTGACCGCTACCTGGCCACTGTCCACCCC
ATCTCTTCCACGAAGTTCCGGAAGCCCTCTGTGGCCACCCTGGTGATCTGC
CTCCTGTGGGCCCTCTCCTTCATCAGCATACCCCTGTGTGGCTGTATGCC
AGACTCATCCCCTTCCCAGGAGGTGCAGTGGGCTGCGGCATACGCCTGCC
35 CAACCCAGACACTGACCTCTACTGGTTCACCCTGTACCAGTTTTTCTCCTGGC

CTTTGCCCTGCCTTTTGTGGTCATCACAGCCGCATACGTGAGGATCCTGCA
GCGCATGACGTCCTCAGTGGCCCCCGCCTCCCAGCGCAGCATCCGGCTGC
GGACAAAGAGGGTGACCCGCACAGCCATCGCCATCTGTCTGGTCTTCTTT
GTGTGCTGGGCACCCTACTATGTGCTACAGCTGACCCAGTTGTCCATCAGC
5 CGCCCGACCCTCACCTTTGTCTACTTATAACAATGCGGCCATCAGCTTGGGC
TATGCCAACAGCTGCCTCAACCCCTTTGTGTACATCGTGCTCTGTGAGACG
TTCCGCAAACGCTTGGTCCTGTCGGTGAAGCCTGCAGCCCAGGGGCAGCT
TCGCGCTGTCAGCAACGCTCAGACGGCTGACGAGGAGAGGACAGAAAGC
AAAGGCACCTGA

10

SEQ. ID. NO. 16: Human short form MCH1R cDNA

ATGGACCTGGAAGCCTCGCTGCTGCCCCACTGGTCCCAATGCCAGCAACAC
CTCTGATGGCCCCGATAACCTCACTTCGGCAGGATCACCTCCTCGCACGG
GGAGCATCTCCTACATCAACATCATCATGCCTTCGGTGTTCGGCACCATCT
15 GCCTCCTGGGCATCATCGGGAACCTCCACGGTCATCTTCGCGGTCTGTGAAG
AAGTCCAAGCTGCACTGGTGCAACAACGTCCCCGACATCTTCATCATCAA
CCTCTCGGTAGTAGATCTCCTCTTTCTCCTGGGCATGCCCTTCATGATCCA
CCAGCTCATGGGCAATGGGGTGTGGCACTTTGGGGAGACCATGTGCACCC
TCATCACGGCCATGGATGCCAATAGTCAGTTCACCAGCACCTACATCCTG
20 ACCGCCATGGCCATTGACCGCTACCTGGCCACTGTCCACCCCATCTCTTCC
ACGAAGTTCGGAAGCCCTCTGTGGCCACCCTGGTGATCTGCCTCCTGTGG
GCCCTCTCCTTCATCAGCATCACCCCTGTGTGGCTGTATGCCAGACTCATC
CCCTTCCCAGGAGGTGCAGTGGGCTGCGGCATACGCCTGCCCAACCCAGA
CACTGACCTCTACTGGTTCACCCTGTACCAGTTTTTCCTGGCCTTTGCCCTG
25 CCTTTTGTGGTCATCACAGCCGCATACGTGAGGATCCTGCAGCGCATGAC
GTCCTCAGTGGCCCCCGCCTCCCAGCGCAGCATCCGGCTGCGGACAAAGA
GGGTGACCCGCACAGCCATCGCCATCTGTCTGGTCTTCTTTGTGTGCTGGG
CACCTACTATGTGCTACAGCTGACCCAGTTGTCCATCAGCCGCCCCGACCC
TCACCTTTGTCTACTTATAACAATGCGGCCATCAGCTTGGGCTATGCCAACA
30 GCTGCCTCAACCCCTTTGTGTACATCGTGCTCTGTGAGACGTTCCGCAAAC
GCTTGGTCCTGTCGGTGAAGCCTGCAGCCCAGGGGCAGCTTCGCGCTGTC
AGCAACGCTCAGACGGCTGACGAGGAGAGGACAGAAAGCAAAGGCACCT
GA

SEQ. ID. NO. 17: Mouse MCH1R cDNA

Nucleic acid sequence start and stop codons are highlighted:

GGCGGTAGAGGAAGACCCTTTTCTGGACTGCGGGGCTCAAGCTCCGGACA
AGGCGGTGGAGGGCGCTGGAGGCTGCCGCAGCCTGCGTGGGTGGACGGG
5 CGCTCCACTCCAGGGAGCAGGCGACCTGCACCGGCTGCATGGATCTGCAA
GCCTCGTTGCTGTCCACTGGCCCCAATGCCAGCAACATCTCCGATGGCCA
GGATAATTTACATTGGCGGGGCCACCTCCTCGCACAAGGAGTGTCTCCT
ACATCAACATCATCATGCCTTCAGTGTTTGGTACCATCTGTCTCCTGGGCA
TTGTGGGAAACTCCACAGTCATTTTTGCCGTGGTGAAGAAATCCAAGCTG
10 CACTGGTGCAGCAACGTCCCTGACATCTTCATCATCAACCTCTCTGTGGTG
GATCTGCTTTTCCTGCTGGGCATGCCTTTCATGATCCACCAGCTCATGGGT
AATGGTGTCTGGCACTTTGGGGAAACCATGTGCACCCTCATCACAGCCAT
GGACGCCAACAGTCAGTTCACCAGCACCTACATCCTGACTGCTATGGCCA
TTGACCGCTACTTGGCCACCGTCCATCCCATCTCCTCCACCAAGTTCCGGA
15 AGCCCTCCATGGCCACCCTGGTGATCTGCCTCCTGTGGGCTCTCTCGTTCA
TTAGCATCACTCCTGTGTGGCTCTATGCCAGGCTTATCCCCTTCCCAGGGG
GTGCTGTGGGCTGTGGCATCCGCCTACCAAACCCAGATACTGATCTTTACT
GGTTCACTCTGTATCAGTTTTTCTGGCCTTCGCCCTTCCGTTTGTGGTCAT
CACTGCTGCGTACGTGAAAATACTACAGCGCATGACGTCTTCGGTGGCCCC
20 CAGCCTCTCAACGCAGCATCCGGCTTCGGACAAAGAGGGTGACCCGCACA
GCCATTGCCATCTGTCTGGTCTTCTTTGTGTGCTGGGCGCCCTACTACGTG
CTGCAGCTGACCCAGTTGTCCATCAGCCGCCCCGACCCTCACATTTCGTCTAC
CTGTACAATGCGGCCATCAGCTTGGGCTATGCCAACAGCTGCCTCAATCC
CTTTGTGTACATAGTACTCTGTGAGACCTTTCGAAAACGCTTGGTGCTGTC
25 GGTGAAGCCCGCGGCCAGGGGCAGCTTCGCACGGTCAGCAATGCTCAGA
CAGCTGACGAGGAGAGGACAGAAAGCAAAGGCACCTGACAATCCCCCCC
GGTCACCTCCAAGTCAGGTCACCGCATCAAACCATGGGGAGAGATACTGA
GATAAACCCGGGGCTACCCTGGGAGGATGCAGAAGCTGGAGGCTGGGGG
CTTGTAGCAAACCACATTCCACGGGGCCCAAAATTGCTAGGGAGGCTTG
30 CAGCCTGGTTTGGGGGGGAAGCCTCAGACTGCAGGGATCCCCTTGACAGA
ATAGAAGCGGAGCAAGAAGGAAAGGGTGGTTTACTGGTTCTCGGGGTCT
GTATCTGTTGGCTCGCATATATCTTTCTCTCAAGGGAAGAAGGCGGAGGT
GCCTAGCTGGGTTCCCTTTAAAACTAGGCAGGGCTAGGATCTGAGCAGCTA
GGGCTCTACTGTGAGACTGGGCAAGCCGAGCGTTCCTCCCATCTCTCAT
35 GGTGTTGATAGAAGGCAGTCTTTCTCCCAAGCTGGTGGATCTCCTGAAGC

ACGCTGCCTGGGCTCCAGCATCCTGTGCGGATTTACGTTCTCTTTAGGGG
ATGCATGTTGACACTGGGGTGTGGGCTCTGAGCCACAGGAGTTTAAAAA
ACCAAAAGAGCTCAGAGTGTCGAGAGAGACCCAATCACCGAGAATGACA
AGGCAACCTGGGGTGGATGTGGATCTTGAACTAATAAAAAGGGGTTTTTC
5 ACAGTGACAGCGACATTCTCTTCATAGGGCACAGCTGTCAGTCTATGGCT
GATCCAGAGCGAGCATCCATGAATTCTGCATGTGCAGGGGTCACTCTAAT
ACCTGATATGTTGGCATCATCTTTGTGCTTGAGCCTTCNCTCCCAAATGG
GAATGAAATAAAGGCAAATTCNCCCCCCCCCAAAAAGGGGNAAAAAA
AAAAAAAAAAAAAAAAAAAA

10

SEQ. ID. NO. 18: Mouse MCH1R genomic DNA

Nucleic acid sequence start and stop codons, as well as intron borders, are highlighted:

GGCGGTAGAGGAAGACCCTTTTCTGGACTGCGGGGCTCAAGCTCCGGACA
15 AGGCGGTGAGGGGCGCTGGAGGCTGCCGCAGCCTGCGTGGGTGGACGGG
CGCTCCACTCCAGGGAGCAGGCGACCTGCACCGGCTGCATGGATCTGCAA
GCCTCGTTGCTGTCCACTGGCCCCAATGCCAGCAACATCTCCGATGGCCA
GGATAATTTACATTGGCGGGTGAGTCGAGTTGGAGTCCTCCCTCCTCCG
GGATGGGTGTGGAAAATGGGAAGGTTTACCTCCCAAGCCAACTGCCTG
20 GGAACTTTATCTTACAGTTCTTGGTGATAAGATCTGCAGTCGGCTTTGCC
TGAAGAGGAAGAGGAGAGGAGGGGACACCAGCTAGGACAGAAGGGGCA
GGGAGGAATAGAGATGGGGCAGAGGCACATTTAGAAACAACAAGGGTTG
GTGACAAGACGTGAGGCAGGCTTGAGGGGAAAGCTTGCTGATGAGTCCCA
AATATGCTTTGCAGGGGGGGGGGGGGGAATCAAGGCTGGAGAAGCAA
25 GCAAGCAAGACAGCAAGACAGCGGGCGGGTAGTATGTGGGAGCCAGCAG
AAGCGCTTTGATTCACCGCTATCCTGGGCTCAATCCTCTGGCCTCGCACTG
GGGAAATGGGGTCTGAGTGGTCCTTGCTGTCTTCTGGCAAAGGCTGCTGG
GAGCAAAAGACTTCACAGGGCGTGAGAGGATTAACCTTTTCTGGTGAATTA
AGCTTCTTGACATTTGCAGAACGTCAATGCCTTAAAATTCTAGCTCTGAAG
30 GAGAAGGGAATGAAGGGGAAAGAGGGAAGGTTGGTGTGGAGAAATTCCC
AAGCTTCTGGGGTGTAAACACAGCTCCAGTCCCTACCCTATTGGGAAAGCC
CAGACTCAGGAGACATGGTCCAAGGAAATCCCTGACAGAAAACCGGGAG
AGGGCAGGGCTGTGGAGCCTGAAACACACCCACACCCATGGTGACAGTC
ACTTCTCACATATGCCTAGGAACCTATCTGAAACCTTTGGCCATCTCTCTC
35 TGAAAAGATGAGGCTGCAAATACACACACACACACACACACACACAC

ACACACACACACACACACACACACACACACACAAATGTCCTTCAAGCC
TTTTTGACAAGGTTTTCTGGTGGATCCCGGGGATATGAAGTTGTTCTCAGC
AGATATCTGGGAGTCTTGACTCCTGGCCCTCTGAGTAAATGGATGAAGCG
AAGAAGAATGGGGTCCTCTGAGTAACAGGTGGATCTAGAAAATCCTATAG
5 GAGTCACCAGGGCACGGTGGAGGAGGGTAAGGTACAGAACTAACAATAG
CCCGAGAAGGGGAAACAGCAGGAGATGATTCCAGAGACGTAGTGACCCC
AAGCTGCAAGGGAAAGCATGAGGGGCCAGCAGGAAGGCCGACATGGCAG
GTTGTCAGCTTCTAGATCGGAAGGCGGGTCACACTTGCTCTTTCTATCCTC
AGGGCCACCTCCTCGCACAAGGAGTGTCTCCTACATCAACATCATCATGC
10 CTTCAGTGTTTGGTACCATCTGTCTCCTGGGCATTGTGGGAAACTCCACAG
TCATTTTTGCCGTGGTGAAGAAATCCAAGCTGCACTGGTGCAGCAACGTC
CCTGACATCTTCATCATCAACCTCTCTGTGGTGGATCTGCTTTTCCTGCTGG
GCATGCCTTTCATGATCCACCAGCTCATGGGTAATGGTGTCTGGCACTTTG
GGGAAACCATGTGCACCCTCATCACAGCCATGGACGCCAACAGTCAGTTC
15 ACCAGCACCTACATCCTGACTGCTATGGCCATTGACCGCTACTTGGCCACC
GTCCATCCCATCTCCTCCACCAAGTTCCGGAAGCCCTCCATGGCCACCCTG
GTGATCTGCCTCCTGTGGGCTCTCTCGTTCATTAGCATCACTCCTGTGTGG
CTCTATGCCAGGCTTATCCCCTTCCCAGGGGGTGCTGTGGGCTGTGGCATC
CGCCTACCAAACCCAGATACTGATCTTTACTGGTTCCTCTGTATCAGTTT
20 TTCCTGGCCTTCGCCCTTCCGTTTGTGGTCATCACTGCTGCGTACGTGAAA
ATACTACAGCGCATGACGTCTTCGGTGGCCCCAGCCTCTCAACGCAGCAT
CCGGCTTCGGACAAAGAGGGTGACCCGCACAGCCATTGCCATCTGTCTGG
TCTTCTTTGTGTGCTGGGCGCCCTACTACGTGCTGCAGCTGACCCAGTTGT
CCATCAGCCGCCGACCCTCACATTCGTCTACCTGTACAATGCGGCCATCA
25 GCTTGGGCTATGCCAACAGCTGCCTCAATCCCTTTGTGTACATAGTACTCT
GTGAGACCTTTCGAAAACGCTTGGTGTCTGTCGGTGAAGCCCGCGGCCAG
GGGCAGCTTCGCACGGTCAGCAATGCTCAGACAGCTGACGAGGAGAGGA
CAGAAAGCAAAGGCACCTGACAATCCCCCCCCGGTCACCTCCAAGTCAGGT
CACCGCATCAAACCATGGGGAGAGATACTGAGATAAACCCGGGGCTACC
30 CTGGGAGGATGCAGAAGCTGGAGGCTGGGGGCTTGTAGCAAACCACATTC
CACGGGGCCCAAAATTGCTAGGGAGGCTTGACAGCCTGGTTTGGGGGGGA
AGCCTCAGACTGCAGGGATCCCCTTGACAGAATAGAAGCGGAGCAAGAA
GGAAAGGGTGGTTTGAAGTGGTTCTCGGGGTCTGTATCTGTTGGCTCGCATA
TATCTTTCTCTCAAGGGAAGAAGGCGGAGGTGCCTAGCTGGGTTCCCTTA
35 AAAGTAGGCAGGGCTAGGATCTGAGCAGCTAGGGCTCTACTGTGAGACTG

GGCAAGCCGAGCGTTCCCTCCCATCTCTCATTGGTGTGATAGAAGGCAG
TCTTTCTCCCAAGCTGGTGGATCTCCTGAAGCACGCTGCCTGGGCTCCAGC
ATCCTGTGCGGATTTACGTTCTCTTTAGGGGATGCATGTTGACACTGGGG
TGTGGGCTCTGAGCCCACAGGAGTTTAAAAAACCAAAGAGCTCAGAGTG
5 TCGAGAGAGACCCAATCACCGAGAATGACAAGGCAACCTGGGGTGGATG
TGGATCTTGAACTAATAAAAAGGGGTTTTACAGTGACAGCGACATTCT
CTTCATAGGGCACAGCTGTCAGTCTATGGCTGATCCAGAGCGAGCATCCA
TGAATTCTGCATGTGCAGGGGTCACTCTAATACCTGATATGTTGGCATCAT
CTTTGTGCTTGAGCCTTCNCTCCCAAATGGGAATGAAATAAAGGCAAAT
10 TCCCNCCCCCCCCAAAAAGGGGNAAAAAAAAAAAAAAAAAAAAAAAAAA
AA

SEQ. ID. NO. 19: Human short form/mouse species chimeric MCH1R

ATGGACCTGGAAGCCTCGCTGCTGCCCACTGGTCCCAATGCCAGCAACAC
15 CTCTGATGGCCCCGATAACCTCACTTCGGCAGGATCACCTCCTCGCACGG
GGAGCATCTCCTACATCAACATCATCATGCCTTCGGTGTTCGGCACCATCT
GCCTCCTGGGCATCATCGGGAACCTCCACGGTCATCTTCGCGGTCTGTGAAG
AAGTCCAAGCTGCACTGGTGCAACAACGTCCCCGACATCTTCATCATCAA
CCTCTCGGTAGTAGATCTCCTCTTTCTCCTGGGCATGCCCTTCATGATCCA
20 CCAGCTCATGGGCAATGGGGTGTGGCACTTTGGGGAGACCATGTGCACCC
TCATCACGGCCATGGATGCCAATAGTCAGTTCACCAGCACCTACATCCTG
ACCGCCATGGCCATTGACCGCTACCTGGCCACTGTCCACCCCATCTCTTCC
ACGAAGTTCCGGAAGCCCTCCATGGCCACCCTGGTGATCTGCCTCCTGTG
GGCTCTCTCGTTCATTAGCATCACTCCTGTGTGGCTCTATGCCAGGCTTAT
25 CCCCTTCCCAGGGGGTGCTGTGGGCTGTGGCATCCGCCTACCAAACCCAG
ATACTGATCTTTACTGGTTCACTCTGTATCAGTTTTCTCGGCCTTCGCCCT
TCCGTTTGTGGTCATCACTGCTGCGTACGTGAAAATACTACAGCGCATGAC
GTCTTCGGTGGCCCCAGCCTCTCAACGCAGCATCCGGCTTCGGACAAAGA
GGGTGACCCGCACAGCCATTGCCATCTGTCTGGTCTTCTTTGTGTGCTGGG
30 CGCCCTACTACGTGCTGCAGCTGACCCAGTTGTCCATCAGCCGCCCCGACC
CTCACATTTCGTCTACCTGTACAATGCGGCCATCAGCTTGGGCTATGCCAAC
AGCTGCCTCAATCCCTTTGTGTACATAGTACTCTGTGAGACCTTTCGAAAA
CGCTTGGTGCTGTCGGTGAAGCCCGCGGCCAGGGGCAGCTTCGCACGGT
CAGCAATGCTCAGACAGCTGACGAGGAGAGGACAGAAAGCAAAGGCACC
35 TGA

SEQ. ID. NO. 20: Human long form/mouse species chimeric MCH1R

ATGTCAGTGGGAGCCATGAAGAAGGGAGTGGGGAGGGCAGTTGGGCTTG
GAGGCGGCAGCGGCTGCCAGGCTACGGAGGAAGACCCCCTTCCCAACTGC
5 GGGGCTTGCGCTCCGGGACAAGGTGGCAGGCGCTGGAGGCTGCCGCAGC
CTGCGTGGGTGGAGGGGAGCTCAGCTCGGTTGTGGGAGCAGGCGACCGG
CACTGGCTGGATGGACCTGGAAGCCTCGCTGCTGCCCCACTGGTCCCAACG
CCAGCAACACCTCTGATGGCCCCGATAACCTCACTTCGGCAGGATCACCT
CCTCGCACGGGGAGCATCTCCTACATCAACATCATCATGCCTTCGGTGTTC
10 GGCACCATCTGCCTCCTGGGCATCATCGGGAAGTCCACGGTCATCTTCGCG
GTCGTGAAGAAGTCCAAGCTGCACTGGTGCAACAACGTCCCCGACATCTT
CATCATCAACCTCTCGGTAGTAGATCTCCTCTTTCTCCTGGGCATGCCCTT
CATGATCCACCAGCTCATGGGCAATGGGGTGTGGCACTTTGGGGAGACCA
TGTGCACCCTCATCACGGCCATGGATGCCAATAGTCAGTTCACCAGCACC
15 TACATCCTGACCGCCATGGCCATTGACCGCTACCTGGCCACTGTCCACCCC
ATCTCTTCCACGAAGTTCGGGAAGCCCTCCATGGCCACCCTGGTGATCTGC
CTCCTGTGGGCTCTCTCGTTCATTAGCATCACTCCTGTGTGGCTCTATGCC
AGGCTTATCCCCTTCCCAGGGGGTGTGTGGGCTGTGGCATCCGCCTACCA
AACCCAGATACTGATCTTTACTGGTTCACCTCTGTATCAGTTTTCTGGCCT
20 TCGCCCTTCCGTTTGTGGTCATCACTGCTGCGTACGTGAAAATACTACAGC
GCATGACGTCTTCGGTGGCCCCAGCCTCTCAACGCAGCATCCGGCTTCGG
ACAAAGAGGGTGACCCGCACAGCCATTGCCATCTGTCTGGTCTTCTTTGTG
TGCTGGGCGCCCTACTACGTGCTGCAGCTGACCCAGTTGTCCATCAGCCGC
CCGACCCTCACATTCGTCTACCTGTACAATGCGGCCATCAGCTTGGGCTAT
25 GCCAACAGCTGCCTCAATCCCTTTGTGTACATAGTACTCTGTGAGACCTTT
CGAAAACGCTTGGTGCTGTTCGGTGAAGCCCGCGGCCAGGGGCAGCTTCG
CACGGTCAGCAATGCTCAGACAGCTGACGAGGAGAGGACAGAAAGCAAA
GGCACCTGA

30 SEQ. ID. NO. 21: *Aequorea victoria* Green Fluorescent Protein (GFP) cDNA

Nucleic acid sequence start and stop codons are highlighted:

TACACACGAATAAAAGATAACAAAGATGAGTAAAGGAGAAGAAGCTTTTC
ACTGGAGTTGTCCCAATTCTTGTGAATTAGATGGTGATGTTAATGGGCAC
AAATTTTCTGTCAAGTGGAGAGGGTGAAGGTGATGCAACATACGGAAAAGT
35 TACCCTTAAATTTATTTGCACTACTGGAAAAGTACCTGTTCCATGGCCAAC

ACTTGTCACTACTTTCTCTTATGGTGTTCATGCTTTTCAAGATACCCAGAT
 CATATGAAACAGCATGACTTTTCAAGAGTGCCATGCCCCGAAGGTTATGT
 ACAGGAAAGAACTATATTTTCAAAGATGACGGGAACTACAAGACACGTG
 CTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATAGAATCGAGTTAAAA
 5 GGTATTGATTTTAAAGAAGATGGAAACATTCTTGGACACAAATTGGAATA
 CAACTATAACTCACACAATGTATACATCATGGCAGACAAACAAAAGAATG
 GAATCAAAGTTAACTTCAAAATTAGACACAACATTGAAGATGGAAGCGTT
 CAACTAGCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGT
 CCTTTTACCAGACAACCATTACCTGTCCACACAATCTGCCCTTTCGAAAGA
 10 TCCCAACGAAAAGAGAGACCACATGGTCCTTCTTGAGTTTGTAAACAGCTG
 CTGGGATTACACATGGCATGGATGAACTATACAAATAAATGTCCAGACTT
 CCAATTGACACTAAAGTGTCCGAACAATTACTAAAATCTCAGGGTTCCTG
 GTTAAATTCAGGCTGAGATATTATTTATATATTTATAGATTCATTAATAATT
 GTATGAATAATTTATTGATGTTATTGATAGAGGTTATTTTCTTATTAAACA
 15 GGCTACTTGGAGTGTATTCTTAATTCTATATTAATTACAATTTGATTGACT
 TGCTCAA

SEQ. ID. NO. 22: EGFP + Linker

Nucleic acid sequence start and stop codons are highlighted and a 12 amino acid

20 linker sequence is denoted in lower case:

gtcgacggtaccgcgggccgggatccatgccacc**ATGGTGAGCAAGGGCGAGGAGCTGTT**
CACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCC
ACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAA
GCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCCGTGCCCTGGC
 25 **CCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTAC**
CCCGACCACATGAAGCAGCAGCACTTCTTCAAGTCCGCCATGCCCGAAGG
CTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGA
CCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAG
CTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGC
 30 **TGGAGTACAACACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAG**
AAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACG
GCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGAC
GGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCT
GAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCG

TGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAAGC
GGCCGC

SEQ. ID. NO. 23: Emerald

5 ATGGTGAGCAAGGGCGAGGAGCTGTTACCGGGGTGGTGCCCATCCTGGT
CGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAG
GGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCAC
CACCGGCAAGCTGCCCCTGCCCTGGCCACCCTCGTGACCACCTTGACCT
ACGGCGTGCAAGTCTCGCCCGCTACCCCGACCACATGAAGCAGCACGAC
10 TTCTTCAAGTCCGCCATGCCCCGAAGGCTACGTCCAGGAGCGCACCATCTTC
TTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGG
GCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAG
GACGGCAACATCCTGGGGCACAAGCTGGAGTACAACATAACAGCCACA
AGGTCTATATCACCGCCGACAAGCAGAAGAACGGCATCAAGGTGAACCTC
15 AAGACCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACT
ACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCCGACAAC
CACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCG
CGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCG
GCATGGACGAGCTGTACAAGTAA

20

SEQ. ID. NO. 24: Topaz

ATGGTGAGCAAGGGCGAGGAGCTGTTACCGGGGTGGTGCCCATCCTGGT
CGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAG
GGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCAC
25 CACCGGCAAGCTGCCCCTGCCCTGGCCACCCTCGTGACCACCTTCGGCT
ACGGCGTGCAAGTCTCGCCCGCTACCCCGACCACATGCGCCAGCACGAC
TTCTTCAAGTCCGCCATGCCCCGAAGGCTACGTCCAGGAGCGCACCATCTTC
TTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGG
GCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAG
30 GACGGCAACATCCTGGGGCACAAGCTGGAGTACAACATAACAGCCACA
ACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACCTC
AAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTA
CCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCCGACAACC
ACTACCTGAGCTACCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGC

GATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGG
CATGGACGAGCTGTACAAGTAA

SEQ. ID. NO. 25: W1B

5 ATGGTGAGCAAGGGCGAGGAGCTGTTACCGGGGTGGTGCCCATCCTGGT
CGAGCTGGACGGCGACGTAAACGGCCACAGGTTACGCGTGTCCGGCGAG
GGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCAC
CACCGGCAAGCTGCCCCTGCCCTGGCCCACCCTCGTGACCACCCTGACCT
GGGGCGTGCAAGTCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGAC
10 TTCTTCAAGTCCGCCATGCCCCGAAGGCTACGTCCAGGAGCGTACCATCTTC
TTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGG
GCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAG
GACGGCAACATCCTGGGGCACAAGCTGGAGTACAACATACATCAGCCACA
ACGTCTATATCACCGCCGACAAGCAGAAGAACGGCATCAAGGCCCACTTC
15 AAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTA
CCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCCGACAACC
ACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGC
GATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGG
CATGGACGAGCTGTACAAGTAA

20

SEQ. ID. NO. 26: Mouse MCH1R-linker-EGFP

Nucleic acid sequence start codon and start and stop codons for mouse MCH1R and EGFP, respectively, as well as intron borders, are highlighted and a 12 amino acid linker sequence is denoted in lower case:

25 ATGGATCTGCAAGCCTCGTTGCTGTCCACTGGCCCCAATGCCAGCAACAT
CTCCGATGGCCAGGATAATTTACATTGGCGGGTGAGTCGAGTTGGAGTC
CTCCCTCCTCCGGGATGGGTGTGGAAAATGGGAAGGTTTCACCTCCCAAG
CCAAACTGCCTGGGAAACTTTATCTTACAGTTCTTGGTGATAAGATCTGCA
GTCGGCTTTGCCTGAAGAGGAAGAGGAGAGGAGGGGACACCAGCTAGGA
30 CAGAAGGGGCAGGGAGGAATAGAGATGGGGCAGAGGCACATTTAGAAAC
AACAAGGGTTGGTGACAAGACGTGAGGCAGGCTTGAGGGGAAAGCTTGC
TGATGAGTCCCAAATATGCTTTGCAGGGGGGGGGGGGGGAATCAAGG
CTGGAGAAGCAAGCAAGCAAGACAGCAAGACAGCGGGCGGGTAGTATGT
GGGAGCCAGCAGAAGCGCTTTGATTACCGCTATCCTGGGCTCAATCCTC
35 TGGCCTCGCACTGGGGAAATGGGGTCTGAGTGGTCCTTGCTGTCTTCTGGC

AAAGGCTGCTGGGAGCAAAAGACTTCACAGGGCGTGAGAGGATTAACCTT
TCTGGTGAATTAAGCTTCTTGACATTTGCAGAACGTCAATGCCTTAAAT
CTAGCTCTGAAGGAGAAGGGAATGAAGGGGAAAGAGGGAAGGTTGGTGT
GGAGAAATTCCCAAGCTTCTGGGGTGTAACACAGCTCCAGTCCCTACCCT
5 ATTGGGAAAGCCCAAGACTCAGGAGACATGGTCCAAGGAAATCCCTGACA
GAAAACCGGGAGAGGGCAGGGCTGTGGAGCCTGAAACACACCCACACC
CATGGTGACAGTCACTTCTCACATATGCCTAGGAACCTATCTGAAACCTT
GGCCATCTCTCTCTGAAAAGATGAGGCTGCAAATACACACACACACAC
ACAAA
10 TGTCTTCAAGCCTTTTTGACAAGGTTTTCTGGTGGATCCCGGGGATATGA
AGTTGTTCTCAGCAGATATCTGGGAGTCTTGACTCCTGGCCCTCTGAGTAA
ATGGATGAAGCGAAGAAGAATGGGGTCCTCTGAGTAACAGGTGGATCTA
GAAAATCCTATAGGAGTCACCAGGGCACGGTGGAGGAGGGTAAGGTACA
GAACTAACAATAGCCCGAGAAGGGGAAACAGCAGGAGATGATTCCAGAG
15 ACGTAGTGACCCCAAGCTGCAAGGGAAAGCATGAGGGGCCAGCAGGAAG
GCCGACATGGCAGGTTGTCAGCTTCTAGATCGGAAGGCGGGTCACACTTG
CTCTTTCTATCCTCAGGGCCACCTCCTCGCACAAGGAGTGTCTCCTACATC
AACATCATCATGCCTTCAGTGTTTGGTACCATCTGTCTCCTGGGCATTGTG
GGAAACTCCACAGTCATTTTTGCCGTGGTGAAGAAATCCAAGCTGCACTG
20 GTGCAGCAACGTCCCTGACATCTTCATCATCAACCTCTCTGTGGTGGATCT
GCTTTTCCTGCTGGGCATGCCTTTCATGATCCACCAGCTCATGGGTAATGG
TGTCTGGCACTTTGGGGAAACCATGTGCACCCTCATCACAGCCATGGACG
CCAACAGTCAGTTCACCAGCACCTACATCCTGACTGCTATGGCCATTGACC
GCTACTTGGCCACCGTCCATCCCATCTCCTCCACCAAGTTCGGGAAGCCCT
25 CCATGGCCACCCTGGTGATCTGCCTCCTGTGGGCTCTCTCGTTTATTAGCA
TCACTCCTGTGTGGCTCTATGCCAGGCTTATCCCCTTCCCAGGGGGTGCTG
TGGGCTGTGGCATCCGCCTACCAAACCCAGATACTGATCTTTACTGGTTCA
CTCTGTATCAGTTTTTCCTGGCCTTCGCCCTTCCGTTTGTGGTTCATCACTGC
TGCGTACGTGAAAATACTACAGCGCATGACGTCTTCGGTGGCCCCAGCCT
30 CTCAACGCAGCATCCGGCTTCGGACAAAGAGGGTGACCCGCACAGCCATT
GCCATCTGTCTGGTCTTCTTTGTGTGCTGGGCGCCCTACTACGTGCTGCAG
CTGACCCAGTTGTCCATCAGCCGCCGACCCTCACATTCGTCTACCTGTAC
AATGCGGCCATCAGCTTGGGCTATGCCAACAGCTGCCTCAATCCCTTTGTG
TACATAGTACTCTGTGAGACCTTTCGAAAACGCTTGGTGTGTCGGTGAA
35 GCGCGCGGCCAGGGGCAGCTTCGCACGGTCAGCAATGCTCAGACAGCTG

ACGAGGAGAGGACAGAAAGCAAAGGCACCgtcgacgggtaccgcgggcccgggatccatcg
ccaccATGGTGAGCAAGGGCGAGGAGCTGTTACACGGGGTGGTGCCCATCC
TGGTCGAGCTGGACGGCGACGTAAACGGGCCACAAGTTCAGCGTGTCCGGC
GAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTG
5 C'ACCACCGGCAAGCTGCCCCGTGCCCTGGCCCCACCCTCGTGACCACCCTGA
CCTACGGCGTGCAAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCAC
GACTTCTTCAAGTCCGCCATGCCCCGAAGGCTACGTCCAGGAGCGCACCAT
CTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCCG
AGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAG
10 GAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACATAACAGCC
ACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAA
CTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACC
ACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGAC
AACCCTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAA
15 GCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTC
TCGGCATGGACGAGCTGTACAAGTAA

SEQ. ID. NO. 27: Mouse MCH1R/EGFP direct fusion

Nucleic acid sequence start codon and start and stop codons for mouse MCH1R and
20 EGFP, respectively, as well as intron borders, are highlighted:
ATGGATCTGCAAGCCTCGTTGCTGTCCACTGGCCCCAATGCCAGCAACAT
CTCCGATGGCCAGGATAATTTACATTGGCGGGTGAGTCGAGTTGGAGTC
CTCCCTCCTCCGGGATGGGTGTGAAAATGGGAAGGTTTACCTCCCAAG
CCAAACTGCCTGGGAACTTTATCTTACAGTTCTTGGTGATAAGATCTGCA
25 GTCGGCTTTGCCTGAAGAGGAAGAGGAGAGGAGGGGACACCAGCTAGGA
CAGAAGGGGCAGGGAGGAATAGAGATGGGGCAGAGGCACATTTAGAAAC
AACAAGGGTTGGTGACAAGACGTGAGGCAGGCTTGAGGGGAAAGCTTGC
TGATGAGTCCCAAATATGCTTTGCAGGGGGGGGGGGGGGGAATCAAGG
CTGGAGAAGCAAGCAAGCAAGACAGCAAGACAGCGGGCGGGTAGTATGT
30 GGGAGCCAGCAGAAGCGCTTTGATTACCGCTATCCTGGGCTCAATCCTC
TGGCCTCGCACTGGGGAAATGGGGTCTGAGTGGTCCTTGCTGTCTTCTGGC
AAAGGCTGCTGGGAGCAAAAGACTTCACAGGGCGTGAGAGGATTAACCTT
TCTGGTGAATTAAGCTTCTTGACATTTGCAGAACGTCAATGCCTTAAAATT
CTAGCTCTGAAGGAGAAGGGAATGAAGGGGAAAGAGGGAAGGTTGGTGT
35 GGAGAAATTCCCAAGCTTCTGGGGTGTAAACACAGCTCCAGTCCCTACCCT

ATTGGGAAAGCCCAGACTCAGGAGACATGGTCCAAGGAAATCCCTGACA
GAAAACCGGGAGAGGGCAGGGCTGTGGAGCCTGAAACACACCCCACACC
CATGGTGACAGTCACTTCTCACATATGCCTAGGAACCTATCTGAAACCTTT
GGCCATCTCTCTCTGAAAAGATGAGGCTGCAAATACACACACACACACAC
5 ACAAA
TGTCCTTCAAGCCTTTTTGACAAGGTTTTCTGGTGGATCCCGGGGATATGA
AGTTGTTCTCAGCAGATATCTGGGAGTCTTGACTCCTGGCCCTCTGAGTAA
ATGGATGAAGCGAAGAAGAATGGGGTCTCTGAGTAACAGGTGGATCTA
GAAAATCCTATAGGAGTCACCAGGGCACGGTGGAGGAGGGTAAGGTACA
10 GAACTAACAATAGCCCGAGAAGGGGAAACAGCAGGAGATGATTCCAGAG
ACGTAGTGACCCCAAGCTGCAAGGGAAAGCATGAGGGGGCCAGCAGGAAG
GCCGACATGGCAGGTTGTCAGCTTCTAGATCGGAAGGCGGGTCACACTTG
CTCTTTCTATCCTCAGGGCCACCTCCTCGCACAAAGGAGTGTCTCCTACATC
AACATCATCATGCCTTCAGTGTTTGGTACCATCTGTCTCCTGGGCATTGTG
15 GGAAACTCCACAGTCATTTTTGCCGTGGTGAAGAAATCCAAGCTGCACTG
GTGCAGCAACGTCCCTGACATCTTCATCATCAACCTCTCTGTGGTGGATCT
GCTTTTCCTGCTGGGCATGCCTTTCATGATCCACCAGCTCATGGGTAATGG
TGTCTGGCACTTTGGGGAAACCATGTGCACCCTCATCACAGCCATGGACG
CCAACAGTCAGTTCACCAGCACCTACATCCTGACTGCTATGGCCATTGACC
20 GCTACTTGGCCACCGTCCATCCCATCTCCTCCACCAAGTTCCGGAAGCCCT
CCATGGCCACCCTGGTGATCTGCCTCCTGTGGGCTCTCTCGTTCATTAGCA
TCACTCCTGTGTGGCTCTATGCCAGGCTTATCCCCTTCCCAGGGGGTGCTG
TGGGCTGTGGCATCCGCCTACCAAACCCAGATACTGATCTTTACTGGTTCA
CTCTGTATCAGTTTTTCTGGCCTTCGCCCTTCCGTTTGTGGTCATCACTGC
25 TGGGTACGTGAAAATACTACAGCGCATGACGTCTTCGGTGGCCCCAGCCT
CTCAACGCAGCATCCGGCTTCGGACAAAGAGGGTGACCCGCACAGCCATT
GCCATCTGTCTGGTCTTCTTTGTGTGCTGGGCGCCCTACTACGTGCTGCAG
CTGACCCAGTTGTCCATCAGCCGCCCCGACCCTCACATTCTGTCTACCTGTAC
AATGCGGCCATCAGCTTGGGCTATGCCAACAGCTGCCTCAATCCCTTTGTG
30 TACATAGTACTCTGTGAGACCTTTCGAAAACGCTTGGTGCTGTCCGTGAA
GCCCCGCGCCCCAGGGGCAGCTTCGCACGGTCAGCAATGCTCAGACAGCTG
ACGAGGAGAGGACAGAAAGCAAAGGCACCATGGTGAGCAAGGGCGAGG
AGCTGTTCAACGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTA
AACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCT
35 ACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCCGTG

CCCTGGCCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAAGTGCTTCAG
CCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGC
CCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAAC
TACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACC
5 GCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGG
GCACAAGCTGGAGTACAACAGCCACAACGTCTATATCATGGCCG
ACAAGCAGAAGAACGGCATCAAGGTGAACCTCAAGATCCGCCACAACAT
CGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCC
ATCGGCGACGGCCCCGTGCTGCTGCCCCGACAACCACTACCTGAGCACCCA
10 GTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGC
TGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTAC
AAGTAA

SEQ. ID. NO. 28: Human short form/mouse species chimeric MCH1R-linker-

15 EGFP

Nucleic acid sequence start codon and start and stop codons for mouse MCH1R and EGFP, respectively, are highlighted and a 12 amino acid linker sequence is denoted in lower case:

ATGGACCTGGAAGCCTCGCTGCTGCCCCACTGGTCCCAATGCCAGCAACAC
20 CTCTGATGGCCCCGATAACCTCACTTCGGCAGGATCACCTCCTCGCACGG
GGAGCATCTCCTACATCAACATCATCATGCCTTCGGTGTTCGGCACCATCT
GCCTCCTGGGCATCATCGGGAAGTCCACGGTCATCTTCGCGGTCTGTAAG
AAGTCCAAGCTGCACTGGTGCAACAACGTCCCCGACATCTTCATCATCAA
CCTCTCGGTAGTAGATCTCCTCTTTCTCCTGGGCATGCCCTTCATGATCCA
25 CCAGCTCATGGGCAATGGGGTGTGGCACTTTGGGGAGACCATGTGCACCC
TCATCACGGCCATGGATGCCAATAGTCAGTTCACCAGCACCTACATCCTG
ACCGCCATGGCCATTGACCGCTACCTGGCCACTGTCCACCCCATCTCTTCC
ACGAAGTTCCGGAAGCCCTCCATGGCCACCCTGGTGATCTGCCTCCTGTG
GGCTCTCTCGTTCATTAGCATCACTCCTGTGTGGCTCTATGCCAGGCTTAT
30 CCCCTTCCCAGGGGGTGTGTGGGCTGTGGCATCCGCCTACCAAACCCAG
ATACTGATCTTTACTGGTTCCTCTGTATCAGTTTTCTTGGCCTTCGCCCT
TCCGTTTGTGGTCATCACTGCTGCGTACGTGAAAATACTACAGCGCATGAC
GTCTTCGGTGGCCCCAGCCTCTCAACGCAGCATCCGGCTTCGGACAAAGA
GGGTGACCCGCACAGCCATTGCCATCTGTCTGGTCTTCTTTGTGTGCTGGG
35 CGCCCTACTACGTGCTGCAGCTGACCCAGTTGTCCATCAGCCGCCCGACC

CTCACATTCTGTCTACCTGTACAATGCGGCCATCAGCTTGGGCTATGCCAAC
 AGCTGCCTCAATCCCTTTGTGTACATAGTACTCTGTGAGACCTTTCGAAAA
 CGCTTGGTGCTGTTCGGTGAAGCCCGCGGCCAGGGGCAGCTTCGCACGGT
 CAGCAATGCTCAGACAGCTGACGAGGAGAGGACAGAAAGCAAAGGCACC
 5 gtcgacgtaccgcgggcccgatccatcgccaccATGGTGAGCAAGGGCGAGGAGCTGTT
 CACCGGGGTGGTGCCCATCCTGGTTCGAGCTGGACGGCGACGTAAACGGCC
 ACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAA
 GCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCCGTGCCCTGGC
 CCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTAC
 10 CCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCCGAAGG
 CTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGA
 CCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAG
 CTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGC
 TGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAG
 15 AAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACG
 GCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGGCGAC
 GGCCCCGTGCTGCTGCCCCGACAACCACTACCTGAGCACCCAGTCCGCCCT
 GAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCG
 TGACCGCCGCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAA

20

SEQ. ID. NO. 29: Human long form/mouse species chimeric MCH1R-linker-EGFP

Nucleic acid sequence start codon and start and stop codons for mouse MCH1R and EGFP, respectively, are highlighted and a 12 amino acid linker sequence is denoted in

25 lower case:

ATGTCAGTGGGAGCCATGAAGAAGGGAGTGGGGAGGGCAGTTGGGCTTG
 GAGGCGGCAGCGGCTGCCAGGCTACGGAGGAAGACCCCTTCCCAACTGC
 GGGGCTTGCGCTCCGGGACAAGGTGGCAGGCGCTGGAGGCTGCCGCAGC
 CTGCGTGGGTGGAGGGGAGCTCAGCTCGGTTGTGGGAGCAGGCGACCGG
 30 CACTGGCTGGATGGACCTGGAAGCCTCGCTGCTGCCCCACTGGTCCCAACG
 CCAGCAACACCTCTGATGGCCCCGATAACCTCACTTCGGCAGGATCACCT
 CCTCGCACGGGGAGCATCTCCTACATCAACATCATCATGCCTTCGGTGTTT
 GGCACCATCTGCCTCCTGGGCATCATCGGGAACCTCCACGGTCATCTTCGCG
 GTCGTGAAGAAGTCCAAGCTGCACTGGTGCAACAACGTCCCCGACATCTT
 35 CATCATCAACCTCTCGGTAGTAGATCTCCTCTTCTCCTGGGCATGCCCTT

CATGATCCACCAGCTCATGGGCAATGGGGTGTGGCACTTTGGGGAGACCA
 TGTGCACCCTCATCACGGCCATGGATGCCAATAGTCAGTTCACCAGCACC
 TACATCCTGACCGCCATGGCCATTGACCGCTACCTGGCCACTGTCCACCCC
 ATCTCTTCCACGAAGTTCGGGAAGCCCTCCATGGCCACCCTGGTGATCTGC
 5 CTCCTGTGGGCTCTCTCGTTCATTAGCATCACTCCTGTGTGGCTCTATGCC
 AGGCTTATCCCCTTCCCAGGGGGTGTGTGGGCTGTGGCATCCGCCTACCA
 AACCCAGATACTGATCTTTACTGGTTCACCTCTGTATCAGTTTTTCCTGGCCT
 TCGCCCTTCCGTTTGTGGTCATCACTGCTGCGTACGTGAAAATACTACAGC
 GCATGACGTCTTCGGTGGCCCCAGCCTCTCAACGCAGCATCCGGCTTCGG
 10 ACAAAGAGGGTGACCCGCACAGCCATTGCCATCTGTCTGGTCTTCTTTGTG
 TGCTGGGCGCCCTACTACGTGCTGCAGCTGACCCAGTTGTCCATCAGCCGC
 CCGACCCTCACATTCGTCTACCTGTACAATGCGGCCATCAGCTTGGGCTAT
 GCCAACAGCTGCCTCAATCCCTTTGTGTACATAGTACTCTGTGAGACCTTT
 CGAAAACGCTTGGTGCTGTTCGGTGAAGCCCGCGGCCAGGGGCAGCTTCG
 15 CACGGTCAGCAATGCTCAGACAGCTGACGAGGAGAGGACAGAAAGCAAA
 GGCACCgtcgacggtaccgcgggcccgatccatcgccaccATGGTGAGCAAGGGCGAGGA
 GCTGTTACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAA
 ACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTAC
 GGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCC
 20 CTGGCCACCCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCC
 GCTACCCCGACCACATGAAGCAGCAGACTTCTTCAAGTCCGCCATGCC
 GAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTA
 CAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGC
 ATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGC
 25 ACAAGCTGGAGTACAACAGCCACAACGTCTATATCATGGCCGAC
 AAGCAGAAGAACGGCATCAAGGTGAAGTTCAGATCCGCCACAACATCG
 AGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCATC
 GGCGACGGCCCCGTGCTGCTGCCCCGACAACCACTACCTGAGCACCCAGTC
 CGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGG
 30 AGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAG
 TAA

Example 2: Generation of Chimeric and Fusion Proteins

DNA vectors encoding fusion proteins between a MCH-R receptor
 35 (MCH1R) and several different superbright variants of Green Fluorescent Protein

(GFP) were generated. GFP variants were fused either via a 12 amino acid linker: TCGACGGTACCGCGGGCCCGGGATCCATCGCCACC (SEQ. ID. NO. 30), amino acid sequence: VDGTAGPGSIAT (SEQ. ID. NO. 31) (linker fusions) or directly to the C-terminus of MCH1R (direct fusions).

5

Mouse MCH1R-linker-GFP Variant Fusion Constructs

Initially, mouse MCH1R was fused in frame via the linker to Enhanced Green Fluorescent Protein (EGFP). MCH1R was PCR-amplified (95°C for 5 minutes; 95°C for 30 seconds, 60°C for 45 seconds, 68°C for 3.5 minutes, for 15 cycles; 68°C for 7 minutes) from a full-length mouse MCH1R genomic DNA lambda clone utilizing a high fidelity polymerase mix (Expand High Fidelity PCR System from Boehringer Mannheim) and PCR primers [MCH1R (Eco RI) 5': GCGAATTCACCATGGATCTGCAAGCCTCG (SEQ. ID. NO. 32), MCH1R (Sal I) 3': GCGTCGACGGTGCCTTTGCTTTCTGTCC (SEQ. ID. NO. 33)] that generated
15 Eco RI and Sal I enzymatic restriction sites at the N- and C-terminus, respectively. The MCH1R N-terminal PCR primer was also designed to introduce a Kozak consensus sequence for translation which contained an Nco I site (5'-ACCATGG-3'), and the MCH1R C-terminal PCR primer was also designed to eliminate the endogenous stop codon present in the mouse MCH1R gene. The resulting PCR
20 product was phenol/chloroform extracted, restriction digested with Eco RI and Sal I, gel purified, and subcloned in frame into the multicloning site of Clontech's pEGFP-N3 vector between Eco RI and Sal I sites. Several resulting clones for this construct were sequenced to identify a clone with an entirely correct nucleotide sequence. This clone was named mMCH1R-I-EGFP for mouse MCH1R-linker-EGFP.

25

An approximately 760 bp Sal I to Not I fragment of mMCH1R-I-EGFP was excised, gel purified, and subcloned into the multicloning site of pBluescript (SK+) (Stratagene) between Sal I and Not I sites. An approximately 710 bp Nco I to Bsr G1 fragment of EGFP was excised from the resulting pBluescript-EGFP vector and replaced with the corresponding Nco I to Bsr G1 fragment of either Emerald,
30 Topaz, or W1B (other superbright GFP variants), which were excised from vectors pRSET-Emerald, pRSET-Topaz, and pRSET-W1B, respectively. pRSET-Emerald, pRSET-Topaz, and pRSET-W1B were obtained from Aurora Biosciences Co. Sal I to Not I fragments containing either Emerald, Topaz, or W1B were excised from the resulting pBluescript-Emerald, pBluescript-Topaz, and pBluescript-W1B vectors,
35 respectively. Appropriate fragments were gel purified and subcloned into mMCH1R-

l-EGFP digested with Sal I and Not I, replacing the Sal I to Not I EGFP fragment with the corresponding Sal I to Not I fragment from either Emerald, Topaz, or W1B. Several clones for each construct were sequenced to confirm the presence of the appropriate GFP variant. The resulting vectors were named mMCH1R-l-Emerald, mMCH1R-l-Topaz, and mMCH1R-l-W1B for mouse MCH1R-linker-Emerald, mouse MCH1R-linker-Topaz, and mouse MCH1R-linker-W1B, respectively.

Mouse MCH1R/GFP Variant Direct Fusion Constructs

A two step PCR strategy was employed to generate the direct fusion constructs. First, mouse MCH1R, EGFP, and Emerald were PCR-amplified from a full-length mouse MCH1R genomic DNA lambda clone, Clontech's pEGFP-N3 vector, and Aurora's pRSET-Emerald vector, respectively. Mouse MCH1R was PCR-amplified according to the previously mentioned conditions utilizing the same N-terminal PCR primer [MCH1R (Eco RI) 5': GCGAATTCACCATGGATCTGCA AGCCTCG (SEQ. ID. NO. 32)], but in this case a different C-terminal PCR primer was employed. The C-terminal PCR primer [MCH1R (EGFP/Emerald) 3': CCTTGCTCACCATGGTGCCTTTGCTTTCTGTCC (SEQ. ID. NO. 34)] eliminated the endogenous stop codon of mouse MCH1R as before and introduced a region of nucleotide sequence complementary to the nucleotide sequence of the N-terminus of EGFP.

EGFP and Emerald were PCR-amplified (95°C for 5 minutes; 95°C for 30 seconds, 60°C for 45 seconds, 68°C for 1.5 minutes, for 15 cycles; 68°C for 7 minutes) separately with a high fidelity polymerase mix (Advantage HF-2 from Clontech) from their respective templates utilizing a common N-terminal PCR primer [EGFP/Emerald (MCH1R) 5': CAGAAAGCAAAGGCACCATGGTGAGCAA GGGCGAGGAGC (SEQ. ID. NO. 35)] that generated a region of nucleotide sequence complementary to the C-terminus of mouse MCH1R and C-terminal PCR primers [EGFP 3': GGCGGATCCTCTAGAGTCGCGGCC (SEQ. ID. NO. 36), or Emerald (EGFP) 3': GCTCTAGAGTCGCGGCCGCTTACTTGACAGCTCGTCC (SEQ. ID. NO. 37)] that generated a Not I site at the C-terminus. The resulting PCR products were electrophoresed on an agarose gel and the appropriate fragments were gel purified.

In a second PCR step, PCR reactions were set up between the previously generated mouse MCH1R and EGFP, or mouse MCH1R and Emerald PCR products. Following an initial 5 minute denaturation step at 95°C, two rounds of

thermocycling (95°C for 30 seconds, 60°C for 45 seconds, 68°C for 4 minutes) were performed in the absence of PCR primers. This allowed the mouse MCH1R and GFP variants to anneal at their complementary regions and to be filled in by the high fidelity polymerase mix (Expand High Fidelity PCR System from Boehringer
5 Mannheim), yielding double stranded template DNA.

Subsequently, the common N-terminal mouse MCH1R [MCH1R (Eco RI) 5': GCGAATTCACCATGGATCTGCAAGCCTCG (SEQ. ID. NO. 32)] and appropriate C-terminal PCR primers [EGFP 3': GGCGGATCCTCTAGAGTC GCGGCC (SEQ. ID. NO. 36) or Emerald (EGFP) 3': GCTCTAGAGTCGCGG
10 CCGCTTACTTGTACAGCTCGTCC (SEQ. ID. NO. 37)] were added to the reactions and thermocycling was continued for an additional fifteen cycles followed by a final extension at 68°C for 7 minutes. The resulting PCR products were phenol/chloroform extracted, restriction digested with Eco RI and Not I, electrophoresed on an agarose gel, and appropriate fragments were gel purified.

15 These Eco RI to Not I fragments represent direct fusions between either mouse MCH1R and EGFP, or mouse MCH1R and Emerald. Clontech's pEGFP-N3 vector was restriction digested with Eco RI and Not I liberating an approximately 780 bp Eco RI to Not I EGFP fragment. This restriction digest was electrophoresed on an agarose gel and the approximately 3.9 Kb pEGFP-N3 vector
20 backbone was gel purified. Eco RI to Not I mouse MCH1R/EGFP or mouse MCH1R/Emerald direct fusion fragments were subcloned into the pEGFP-N3 vector backbone between Eco RI and Not I sites. Several resulting clones for each of these two constructs were sequenced to identify clones with correct nucleotide sequence; however, no clones with entirely correct nucleotide sequences were identified.
25 Fortunately, several clones for each of the two constructs only had nucleotide mismatches in the intron region of mouse MCH1R, and therefore, were not expected to effect the functionality of the resulting fusion proteins. These clones were named mMCH1R/EGFP and mMCH1R/Emerald for mouse MCH1R/EGFP direct fusion and mouse MCH1R/Emerald direct fusion, respectively.

30

Human Short and Long Form/Mouse Species Chimeric

MCH1R-linker-GFP Variant Fusion Constructs

The initial mouse MCH1R-linker-GFP variant fusion constructs were modified to generate both human short form and human long form/mouse species

chimeric MCH1R-linker-GFP variant fusion constructs. An approximately 1.7 kb Hind III to Bsp EI fragment of the mouse MCH1R gene containing exon 1, the intron, and 127 amino acids of exon 2 was excised from the various mouse MCH1R-linker-GFP variant fusion constructs and replaced by either an approximately 470 bp Hind
5 III to Bsp EI fragment from the wild-type human MCH1R short form or an approximately 670 bp Hind III to Bsp EI fragment from the wild-type human MCH1R long form.

Several clones for each construct were sequenced to confirm the presence of the N-terminal region of either the human MCH1R short or long forms.
10 These clones were named hshort/mMCH1R-l-GFP variant or hlong/mMCH1R-l-GFP variant for human short form/mouse species chimeric MCH1R-linker-GFP variant and human long form/mouse species chimeric MCH1R-linker-GFP variant, respectively.

15 Example 3: Functional Evaluation of MCH1R/GFP Variant Fusion Proteins

Both HEK293 Aequorin (National Institutes of Health) and CHO mammalian cell lines were transiently transfected with the various MCH1R/GFP variant fusion constructs, as well as the appropriate control constructs. Transfection was performed using Lipofectamine 2000 (Gibco BRL) per the manufacturer
20 recommended protocol. Approximately 48 hours after transfection cells were harvested, stimulated with various concentrations of human MCH, and assayed for either aequorin bioluminescence (HEK293 Aequorin cells) or cAMP production (CHO cells). Aequorin bioluminescence is a representative measure of intracellular Ca^{2+} mobilization. cAMP production was measured with the Adenylyl Cyclase
25 Activation FlashPlate Assay (NEN Life Science Products, Inc.).

Following transient transfection of the mMCH1R-linker-EGFP construct (MCH-R-l-EGFP) into HEK293 Aequorin cells, the resulting fusion protein exhibited functional activity comparable to that of the wild-type human MCH1R short form (MCH-R wt). By this functional assay, the EC₅₀ value for mMCH1R-l-EGFP
30 was nearly identical to that of the wild-type human short form receptor (Figure 1).

Following transient transfections of the mMCH1R-l-EGFP and mMCH1R/EGFP fusion constructs into CHO cells, the resulting fusion proteins exhibited functional activity comparable to that of the wild-type human MCH1R short form. By this functional assay, the EC₅₀ values for mMCH1R-l-EGFP and
35 mMCH1R/EGFP were comparable to that of the wild-type human receptor (Table 1).

Transient transfections with the corresponding Emerald constructs yielded similar results (data not shown).

Table 1

5

Receptor	EC ₅₀ (nM)
Wild-type Human MCH1R Short Form	2.166
Mouse MCH1R/EGFP	0.819
Mouse MCH1R-l-EGFP	3.199

Following transient transfections of the human short form/mouse species chimeric MCH1R-l-EGFP (HuShort/mMCH1R-l-EGFP) and human long form/mouse species chimeric MCH1R-l-EGFP (HuLong/mMCH1R-l-EGFP) constructs into HEK293 cells, the resulting fusion proteins exhibited functional activity comparable to that of the wild-type human MCH1R short and long forms, respectively. By this functional assay, the EC₅₀ value for each fusion proteins was nearly identical to that of the corresponding wild-type human receptor (Table 2).

15

Table 2

Receptor	EC ₅₀ (nM)
Wild-type Human MCH1R Short Form	22.27
HuShort/mMCH1R-l-EGFP	19.54
Wild-type Human MCH1R Long Form	196.7
HuLong/mMCH1R-l-EGFP Form	217.5

Following transient transfections of the human short form/mouse species chimeric MCH1R-l-EGFP (HuShort/mMCH1R-l-EGFP) and human long form/mouse species chimeric MCH1R-l-EGFP (HuLong/mMCH1R-l-EGFP) constructs into CHO cells, the resulting fusion proteins exhibited functional activity comparable to or less than that of the wild-type human MCH1R short and long forms, respectively (Table 3). By this functional assay, the EC₅₀ value for the human short form/mouse species chimeric MCH1R-l-EGFP fusion protein was comparable to that of the corresponding wild-type human receptor, whereas, the human long form/mouse

25

species chimeric MCH1R-1-EGFP fusion protein had an EC₅₀ value approximately 7.5-fold higher than that of its corresponding wild-type control.

Table 3

Receptor	EC ₅₀ (nM)
Wild-type Human MCH1R Short Form	1.029
Wild-type Human MCH1R Long Form	1.515
HuShort/mMCH1R-1-EGFP	1.565
HuLong/mMCH1R-1-EGFP	11.580

Transient expression of all the MCH1R/GFP variant fusion proteins that underwent functional evaluation resulted in fluorescence primarily associated with the plasma membrane in both HEK293 and CHO cells (data not shown). This pattern of fluorescence is consistent with a predominant membrane associated localization.

Example 4: Generation of Stable Cell Lines

Wild-type CHO cells were transfected using SuperFect (Qiagen) and either mouse MCH-1R-EGFP or human short/mouse species chimeric MCH-1R-EGFP. Forty-eight hours after transfection, transfected cells were subjected to positive selection for approximately ten days in media containing G418. Following selection, MCH-1R-EGFP expressing CHO cells were bulk sorted by Fluorescence Assisted Cell Sorting (FACS) for one or two rounds on the basis of fluorescence intensity to increase the population of cells expressing EGFP. Following bulk sorts, individual clones of varying fluorescence intensities were isolated by FACS and expanded.

Fluorometric Microvolume Assay Technology (FMAT) was initially employed to screen a large number of stable clones by whole cell binding with a fluorescently labeled MCH derivative (SymJz-MCH, PE Biosystems) to identify those clones with good specific binding windows. Several clones exhibiting specific binding windows greater than 3-fold were further evaluated for MCH binding with the SPA-based Binding Assay. Cells from individual clones were dissociated in enzyme free dissociation media and cell membranes were prepared and subsequently tested for

their ability to bind [125 I]Phe 13 -Tyr 19 -MCH in the presence of human MCH. CHO cell lines expressing either mouse MCH-1R-EGFP or human short/mouse species chimeric MCH-1R-EGFP (Figure 4) displayed IC₅₀ values with MCH that were indistinguishable from the corresponding IC₅₀ values obtained with a CHO cell line expressing the wild-type human short isoform of MCH-1R.

The functional activity of these clones was evaluated with the cAMP Flashplate Assay (Figures 2 and 3). CHO cell lines expressing either mouse MCH-1R-EGFP (Figure 2) or human short/mouse species chimeric MCH-1R-EGFP (Figure 3) displayed EC₅₀ values with human MCH that were indistinguishable from the EC₅₀ value obtained with a CHO cell line expressing the wild-type human short isoform of MCH-1R.

The subcellular localization of the MCH-1R-EGFP fusion proteins were determined by confocal microscopy utilizing EGFP fluorescence as a marker for MCH-1R expression. CHO cell lines stably expressing either mouse MCH-1R-EGFP or human short/mouse species chimeric MCH-1R-EGFP displayed EGFP fluorescence primarily associated with the plasma membrane, demonstrating that these MCH-1R-EGFP fusion proteins are primarily associated with the plasma membrane.

Other embodiments are within the following claims. While several embodiments have been shown and described, various modifications may be made without departing from the spirit and scope of the present invention.

WHAT IS CLAIMED IS:

1. A fusion protein comprising:
 - a) a melanin concentrating hormone receptor polypeptide
5 region comprising a sequence selected from the group consisting of: SEQ. ID. NO. 1, SEQ. ID. NO. 2, SEQ. ID. NO. 3, SEQ. ID. NO. 4, and SEQ. ID. NO. 5; and
 - b) a fluorescent polypeptide region joined directly, or
10 though a linker, to the carboxy side of said melanin concentrating hormone receptor polypeptide region.
2. The protein of claim 1, wherein said fluorescent polypeptide
15 region consists of an amino acid sequence selected from the group consisting of SEQ. ID. NO. 6, SEQ. ID. NO. 7, SEQ. ID. NO. 8, SEQ. ID. NO. 9, and SEQ. ID. NO. 10.
3. The protein of claim 2, wherein said melanin concentrating
20 hormone polypeptide region consists of a sequence selected from the group consisting of: SEQ. ID. NO. 1, SEQ. ID. NO. 2, SEQ. ID. NO. 3, SEQ. ID. NO. 4, and SEQ. ID. NO. 5.
4. The protein of claim 3, wherein said protein consists essentially
25 of said melanin concentrating hormone receptor polypeptide region and said fluorescent polypeptide region.
5. The protein of claim 4, wherein said protein consists of the
30 amino acid sequence of SEQ. ID. NO. 11 or SEQ. ID. NO. 12.
6. The protein of claim 1, wherein said melanin concentrating
hormone polypeptide region is a chimeric polypeptide comprising (a) an MCH
binding region from a first species and (b) a transmembrane and intracellular domain
35 region from a second species joined directly, or though a linker, to the carboxy side of said MCH binding region.
7. The protein of claim 6, wherein said fluorescent polypeptide
region consists of an amino acid sequence selected from the group consisting of: SEQ.
ID. NO. 6, SEQ. ID. NO. 7, SEQ. ID. NO. 8, SEQ. ID. NO. 9, and SEQ. ID. NO. 10.

8. The protein of claim 7, wherein said protein consists of the amino acid sequence of SEQ. ID. NO. 13 or SEQ. ID. NO. 14.

5 9. A chimeric melanin concentrating hormone protein comprising:
a) a melanin concentrating hormone binding region
characteristic of a human melanin concentrating hormone receptor;
b) a transmembrane domain characteristic of a non-human
melanin concentrating hormone receptor; and
10 c) an intracellular domain characteristic of a non-human
melanin concentrating hormone receptor.

10. The protein of claim 9, wherein said protein comprises a melanin concentrating hormone receptor polypeptide having a sequence similarity of
15 at least 75% with either SEQ. ID. NO. 4 or SEQ. ID. NO. 5.

11. The protein of claim 10, wherein said protein comprises the sequence of SEQ. ID. NO. 4 or SEQ. ID. NO. 5.

20 12. The protein of claim 11, wherein said protein consists of the sequence of SEQ. ID. NO. 4 or SEQ. ID. NO. 5.

13. A nucleic acid comprising a nucleotide sequence encoding for the protein of claim 1.
25

14. The nucleic acid of claim 13, wherein said nucleotide sequence is a contiguous sequence.

15. The nucleic acid of claim 13, wherein said nucleotide sequence
30 is selected from the group consisting of SEQ. ID. NO. 26, SEQ. ID. NO. 27, SEQ. ID. NO. 28 and SEQ. ID. NO. 29.

16. A nucleic acid comprising a nucleotide sequence encoding for the protein of claim 9.
35

17. The nucleic acid of claim 16, wherein said nucleotide sequence is a contiguous sequence.

18. The nucleic acid of claim 16, wherein said nucleotide sequence is selected from the group consisting of SEQ. ID. NO. 19 and SEQ. ID. NO. 20.

19. An expression vector comprising the nucleic acid of claim 13.

20. An expression vector comprising the nucleic acid of claim 16.

21. A recombinant cell comprising the nucleic acid of claim 13.

22. The recombinant cell of claim 21, wherein said nucleic acid is present in an expression vector.

23. The recombinant cell of claim 21, wherein said nucleic acid is present in the genome of said cell.

24. A recombinant cell comprising the nucleic acid of claim 16.

25. The recombinant cell of claim 24, wherein said nucleic acid is present in an expression vector.

26. The recombinant cell of claim 24, wherein said nucleic acid is present in the genome of said cell.

27. A non-human transgenic animal comprising the nucleic acid of claim 13.

28. A non-human transgenic animal comprising the nucleic acid of claim 16.

29. A method for assaying for melanin concentrating hormone receptor active compounds comprising the steps of:

- a) contacting the cell of claim 21 with a test preparation comprising one or more test compounds; and
- b) measuring the effect of said test preparation on one or more melanin concentrating hormone receptor activities.

5

30. A method for assaying for melanin concentrating hormone receptor active compounds comprising the steps of:

- a) contacting the cell of claim 24 with a test preparation comprising one or more test compounds; and
- b) measuring the effect of said test preparation on one or more melanin concentrating hormone receptor activities.

10

1/3

mMCH1R-I-EGFP Aequorin Assay

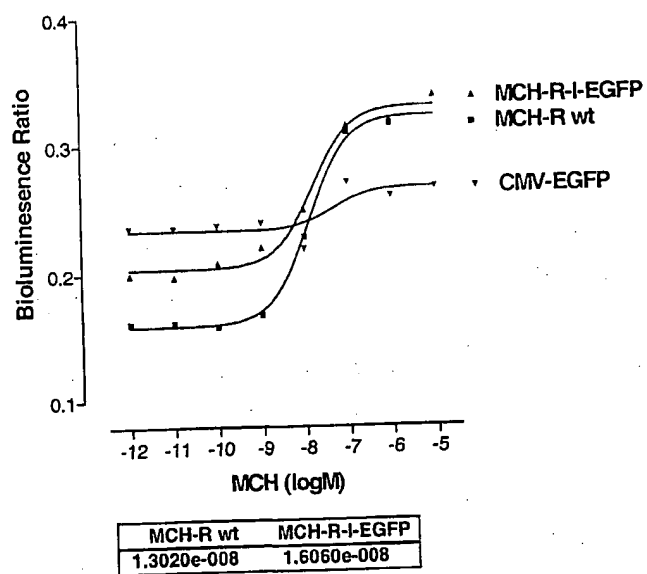


Fig. 1

2/3

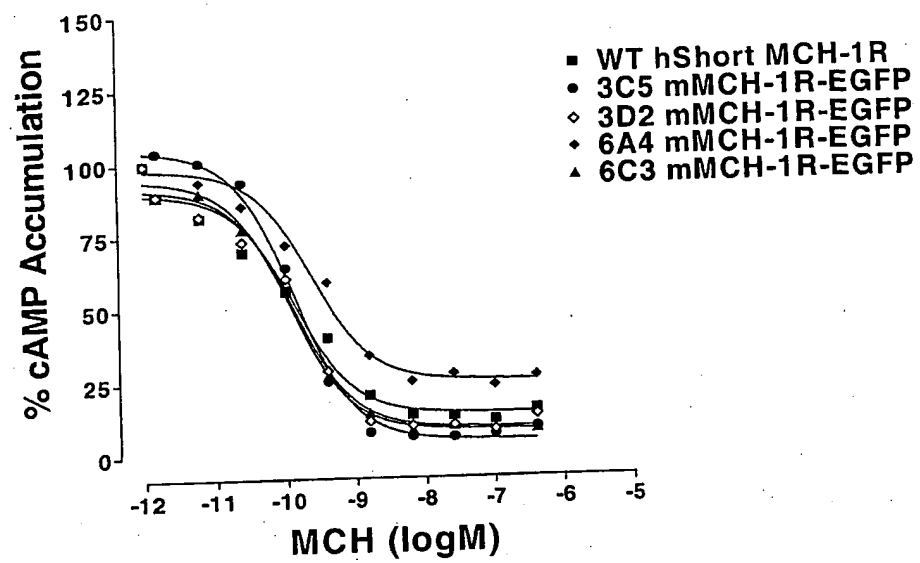


Fig. 2

3/3

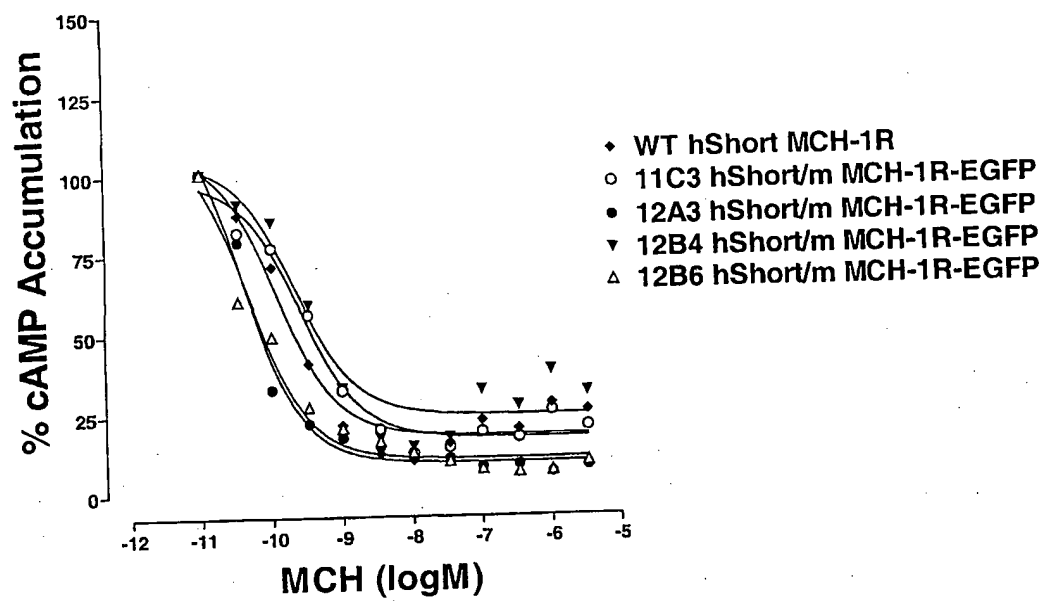


Fig. 3

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 195 200 205
 Thr Leu Tyr Gln Phe Phe Leu Ala Phe Ala Leu Pro Phe Val Val Ile
 210 215 220
 Thr Ala Ala Tyr Val Lys Ile Leu Gln Arg Met Thr Ser Ser Val Ala
 225 230 235 240
 Pro Ala Ser Gln Arg Ser Ile Arg Leu Arg Thr Lys Arg Val Thr Arg
 245 250 255
 Thr Ala Ile Ala Ile Cys Leu Val Phe Phe Val Cys Trp Ala Pro Tyr
 260 265 270
 Tyr Val Leu Gln Leu Thr Gln Leu Ser Ile Ser Arg Pro Thr Leu Thr
 275 280 285
 Phe Val Tyr Leu Tyr Asn Ala Ala Ile Ser Leu Gly Tyr Ala Asn Ser
 290 295 300
 Cys Leu Asn Pro Phe Val Tyr Ile Val Leu Cys Glu Thr Phe Arg Lys
 305 310 315 320
 Arg Leu Val Leu Ser Val Lys Pro Ala Ala Gln Gly Gln Leu Arg Thr
 325 330 335
 Val Ser Asn Ala Gln Thr Ala Asp Glu Glu Arg Thr Glu Ser Lys Gly
 340 345 350
 Thr

<210> 5
 <211> 422
 <212> PRT
 <213> Artificial Sequence
 <220>

<223> Human long form/mouse species chimeric MCH1R

<400> 5
 Met Ser Val Gly Ala Met Lys Lys Gly Val Gly Arg Ala Val Gly Leu
 1 5 10 15
 Gly Gly Gly Ser Gly Cys Gln Ala Thr Glu Glu Asp Pro Leu Pro Asn
 20 25 30
 Cys Gly Ala Cys Ala Pro Gly Gln Gly Gly Arg Arg Trp Arg Leu Pro
 35 40 45
 Gln Pro Ala Trp Val Glu Gly Ser Ser Ala Arg Leu Trp Glu Gln Ala
 50 55 60
 Thr Gly Thr Gly Trp Met Asp Leu Glu Ala Ser Leu Leu Pro Thr Gly
 65 70 75 80
 Pro Asn Ala Ser Asn Thr Ser Asp Gly Pro Asp Asn Leu Thr Ser Ala
 85 90 95
 Gly Ser Pro Pro Arg Thr Gly Ser Ile Ser Tyr Ile Asn Ile Ile Met
 100 105 110
 Pro Ser Val Phe Gly Thr Ile Cys Leu Leu Gly Ile Ile Gly Asn Ser
 115 120 125
 Thr Val Ile Phe Ala Val Val Lys Ser Lys Leu His Trp Cys Asn
 130 135 140
 Asn Val Pro Asp Ile Phe Ile Ile Asn Leu Ser Val Val Asp Leu Leu
 145 150 155 160
 Phe Leu Leu Gly Met Pro Phe Met Ile His Gln Leu Met Gly Asn Gly
 165 170 175
 Val Trp His Phe Gly Glu Thr Met Cys Thr Leu Ile Thr Ala Met Asp
 180 185 190
 Ala Asn Ser Gln Phe Thr Ser Thr Tyr Ile Leu Thr Ala Met Ala Ile
 195 200 205
 Asp Arg Tyr Leu Ala Thr Val His Pro Ile Ser Ser Thr Lys Phe Arg
 210 215 220
 Lys Pro Ser Met Ala Thr Leu Val Ile Cys Leu Leu Trp Ala Leu Ser
 225 230 235 240
 Phe Ile Ser Ile Thr Pro Val Trp Leu Tyr Ala Arg Leu Ile Pro Phe
 245 250 255
 Pro Gly Gly Ala Val Gly Cys Gly Ile Arg Leu Pro Asn Pro Asp Thr
 260 265 270
 Asp Leu Tyr Trp Phe Thr Leu Tyr Gln Phe Phe Leu Ala Phe Ala Leu
 275 280 285
 Pro Phe Val Val Ile Thr Ala Ala Tyr Val Lys Ile Leu Gln Arg Met
 290 295 300
 Thr Ser Ser Val Ala Pro Ala Ser Gln Arg Ser Ile Arg Leu Arg Thr
 305 310 315 320
 Lys Arg Val Thr Arg Thr Ala Ile Ala Ile Cys Leu Val Phe Phe Val
 325 330 335
 Cys Trp Ala Pro Tyr Tyr Val Leu Gln Leu Thr Gln Leu Ser Ile Ser
 340 345 350
 Arg Pro Thr Leu Thr Phe Val Tyr Leu Tyr Asn Ala Ala Ile Ser Leu
 355 360 365
 Gly Tyr Ala Asn Ser Cys Leu Asn Pro Phe Val Tyr Ile Val Leu Cys
 370 375 380
 Glu Thr Phe Arg Lys Arg Leu Val Leu Ser Val Lys Pro Ala Ala Gln
 385 390 395 400
 Gly Gln Leu Arg Thr Val Ser Asn Ala Gln Thr Ala Asp Glu Glu Arg
 405 410 415
 Thr Glu Ser Lys Gly Thr
 420

<210> 6

<211> 238

<212> PRT

<213> Aequorea Victoria

<400> 6
 Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val
 1 5 10 15
 Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
 20 25 30
 Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
 35 40 45
 Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe
 50 55 60
 Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln
 65 70 75 80
 His Asp Phe Phe Lys Ser Ala Met Pro Glu Tyr Val Gln Glu Arg
 85 90 95
 Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
 100 105 110
 Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
 115 120 125
 Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
 130 135 140
 Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly
 145 150 155 160
 Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val
 165 170 175
 Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
 180 185 190
 Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
 195 200 205
 Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
 210 215 220
 Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
 225 230 235

<210> 7
 <211> 239
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> GFP derivative

<400> 7
 Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu
 1 5 10 15
 Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
 20 25 30
 Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
 35 40 45
 Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
 50 55 60
 Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
 65 70 75 80
 Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
 85 90 95
 Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
 100 105 110
 Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
 115 120 125
 Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
 130 135 140
 Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
 145 150 155 160

Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
 165 170 175
 Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
 180 185 190
 Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
 195 200 205
 Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
 210 215 220
 Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys
 225 230 235

<210> 8
 <211> 239
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> GFP derivative

<400> 8
 Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu
 1 5 10 15
 Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
 20 25 30
 Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
 35 40 45
 Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
 50 55 60
 Leu Thr Tyr Gly Val Gln Cys Phe Ala Arg Tyr Pro Asp His Met Lys
 65 70 75 80
 Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
 85 90 95
 Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
 100 105 110
 Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
 115 120 125
 Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
 130 135 140
 Asn Tyr Asn Ser His Lys Val Tyr Ile Thr Ala Asp Lys Gln Lys Asn
 145 150 155 160
 Gly Ile Lys Val Asn Phe Lys Thr Arg His Asn Ile Glu Asp Gly Ser
 165 170 175
 Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
 180 185 190
 Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
 195 200 205
 Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
 210 215 220
 Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys
 225 230 235

<210> 9
 <211> 239
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> GFP derivative

<400> 9
 Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu
 1 5 10 15

Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
 20 25 30
 Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
 35 40 45
 Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
 50 55 60
 Phe Gly Tyr Gly Val Gln Cys Phe Ala Arg Tyr Pro Asp His Met Arg
 65 70 75 80
 Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
 85 90 95
 Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
 100 105 110
 Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
 115 120 125
 Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
 130 135 140
 Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
 145 150 155 160
 Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
 165 170 175
 Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
 180 185 190
 Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Tyr Gln Ser Ala Leu
 195 200 205
 Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
 210 215 220
 Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys
 225 230 235

<210> 10

<211> 239

<212> PRT

<213> Artificial Sequence

<220>

<223> GFP derivative

<400> 10

Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu
 1 5 10 15
 Val Glu Leu Asp Gly Asp Val Asn Gly His Arg Phe Ser Val Ser Gly
 20 25 30
 Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
 35 40 45
 Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
 50 55 60
 Leu Thr Trp Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
 65 70 75 80
 Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
 85 90 95
 Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
 100 105 110
 Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
 115 120 125
 Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
 130 135 140
 Asn Tyr Ile Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln Lys Asn
 145 150 155 160
 Gly Ile Lys Ala His Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
 165 170 175
 Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
 180 185 190

Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
 195 200 205
 Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
 210 215 220
 Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys
 225 230 235

<210> 11
 <211> 604
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Mouse MCH1R-linker-EGFP

<400> 11
 Met Asp Leu Gln Ala Ser Leu Leu Ser Thr Gly Pro Asn Ala Ser Asn
 1 5 10 15
 Ile Ser Asp Gly Gln Asp Asn Phe Thr Leu Ala Gly Pro Pro Pro Arg
 20 25 30
 Thr Arg Ser Val Ser Tyr Ile Asn Ile Ile Met Pro Ser Val Phe Gly
 35 40 45
 Thr Ile Cys Leu Leu Gly Ile Val Gly Asn Ser Thr Val Ile Phe Ala
 50 55 60
 Val Val Lys Lys Ser Lys Leu His Trp Cys Ser Asn Val Pro Asp Ile
 65 70 75 80
 Phe Ile Ile Asn Leu Ser Val Val Asp Leu Leu Phe Leu Leu Gly Met
 85 90 95
 Pro Phe Met Ile His Gln Leu Met Gly Asn Gly Val Trp His Phe Gly
 100 105 110
 Glu Thr Met Cys Thr Leu Ile Thr Ala Met Asp Ala Asn Ser Gln Phe
 115 120 125
 Thr Ser Thr Tyr Ile Leu Thr Ala Met Ala Ile Asp Arg Tyr Leu Ala
 130 135 140
 Thr Val His Pro Ile Ser Ser Thr Lys Phe Arg Lys Pro Ser Met Ala
 145 150 155 160
 Thr Leu Val Ile Cys Leu Leu Trp Ala Leu Ser Phe Ile Ser Ile Thr
 165 170 175
 Pro Val Trp Leu Tyr Ala Arg Leu Ile Pro Phe Pro Gly Gly Ala Val
 180 185 190
 Gly Cys Gly Ile Arg Leu Pro Asn Pro Asp Thr Asp Leu Tyr Trp Phe
 195 200 205
 Thr Leu Tyr Gln Phe Phe Leu Ala Phe Ala Leu Pro Phe Val Val Ile
 210 215 220
 Thr Ala Ala Tyr Val Lys Ile Leu Gln Arg Met Thr Ser Ser Val Ala
 225 230 235 240
 Pro Ala Ser Gln Arg Ser Ile Arg Leu Arg Thr Lys Arg Val Thr Arg
 245 250 255
 Thr Ala Ile Ala Ile Cys Leu Val Phe Phe Val Cys Trp Ala Pro Tyr
 260 265 270
 Tyr Val Leu Gln Leu Thr Gln Leu Ser Ile Ser Arg Pro Thr Leu Thr
 275 280 285
 Phe Val Tyr Leu Tyr Asn Ala Ala Ile Ser Leu Gly Tyr Ala Asn Ser
 290 295 300
 Cys Leu Asn Pro Phe Val Tyr Ile Val Leu Cys Glu Thr Phe Arg Lys
 305 310 315 320
 Arg Leu Val Leu Ser Val Lys Pro Ala Ala Gln Gly Gln Leu Arg Thr
 325 330 335
 Val Ser Asn Ala Gln Thr Ala Asp Glu Glu Arg Thr Glu Ser Lys Gly
 340 345 350
 Thr Val Asp Gly Thr Ala Gly Pro Gly Ser Ile Ala Thr Met Val Ser
 355 360 365

Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu
 370 375 380
 Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu
 385 390 395 400
 Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr
 405 410 415
 Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Thr Tyr
 420 425 430
 Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln His Asp
 435 440 445
 Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile
 450 455 460
 Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe
 465 470 475 480
 Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe
 485 490 495
 Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn
 500 505 510
 Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile Lys
 515 520 525
 Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu
 530 535 540
 Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu
 545 550 555 560
 Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp
 565 570 575
 Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala
 580 585 590
 Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys
 595 600

<210> 12

<211> 592

<212> PRT

<213> Artificial Sequence

<220>

<223> Mouse MCH1R/EGFP

<400> 12

Met Asp Leu Gln Ala Ser Leu Leu Ser Thr Gly Pro Asn Ala Ser Asn
 1 5 10 15
 Ile Ser Asp Gly Gln Asp Asn Phe Thr Leu Ala Gly Pro Pro Pro Arg
 20 25 30
 Thr Arg Ser Val Ser Tyr Ile Asn Ile Ile Met Pro Ser Val Phe Gly
 35 40 45
 Thr Ile Cys Leu Leu Gly Ile Val Gly Asn Ser Thr Val Ile Phe Ala
 50 55 60
 Val Val Lys Lys Ser Lys Leu His Trp Cys Ser Asn Val Pro Asp Ile
 65 70 75 80
 Phe Ile Ile Asn Leu Ser Val Val Asp Leu Leu Phe Leu Leu Gly Met
 85 90 95
 Pro Phe Met Ile His Gln Leu Met Gly Asn Gly Val Trp His Phe Gly
 100 105 110
 Glu Thr Met Cys Thr Leu Ile Thr Ala Met Asp Ala Asn Ser Gln Phe
 115 120 125
 Thr Ser Thr Tyr Ile Leu Thr Ala Met Ala Ile Asp Arg Tyr Leu Ala
 130 135 140
 Thr Val His Pro Ile Ser Ser Thr Lys Phe Arg Lys Pro Ser Met Ala
 145 150 155 160
 Thr Leu Val Ile Cys Leu Leu Trp Ala Leu Ser Phe Ile Ser Ile Thr
 165 170 175

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Pro Val Trp Leu Tyr Ala Arg Leu Ile Pro Phe Pro Gly Gly Ala Val
      180      185      190
Gly Cys Gly Ile Arg Leu Pro Asn Pro Asp Thr Asp Leu Tyr Trp Phe
      195      200      205
Thr Leu Tyr Gln Phe Phe Leu Ala Phe Ala Leu Pro Phe Val Val Ile
      210      215      220
Thr Ala Ala Tyr Val Lys Ile Leu Gln Arg Met Thr Ser Ser Val Ala
      225      230      235
Pro Ala Ser Gln Arg Ser Ile Arg Leu Arg Thr Lys Arg Val Thr Arg
      245      250      255
Thr Ala Ile Ala Ile Cys Leu Val Phe Phe Val Cys Trp Ala Pro Tyr
      260      265      270
Tyr Val Leu Gln Leu Thr Gln Leu Ser Ile Ser Arg Pro Thr Leu Thr
      275      280      285
Phe Val Tyr Leu Tyr Asn Ala Ala Ile Ser Leu Gly Tyr Ala Asn Ser
      290      295      300
Cys Leu Asn Pro Phe Val Tyr Ile Val Leu Cys Glu Thr Phe Arg Lys
      305      310      315
Arg Leu Val Leu Ser Val Lys Pro Ala Ala Gln Gly Gln Leu Arg Thr
      325      330      335
Val Ser Asn Ala Gln Thr Ala Asp Glu Glu Arg Thr Glu Ser Lys Gly
      340      345      350
Thr Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile
      355      360      365
Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser
      370      375      380
Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe
      385      390      395
Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr
      405      410      415
Thr Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met
      420      425      430
Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln
      435      440      445
Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala
      450      455      460
Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys
      465      470      475
Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu
      485      490      495
Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys
      500      505      510
Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly
      515      520      525
Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp
      530      535      540
Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala
      545      550      555
Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu
      565      570      575
Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys
      580      585      590

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<210> 13
 <211> 604
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> MCH1R-linker-EGFP

<400> 13

Met	Asp	Leu	Glu	Ala	Ser	Leu	Leu	Pro	Thr	Gly	Pro	Asn	Ala	Ser	Asn
1				5					10					15	
Thr	Ser	Asp	Gly	Pro	Asp	Asn	Leu	Thr	Ser	Ala	Gly	Ser	Pro	Pro	Arg
		20						25					30		
Thr	Gly	Ser	Ile	Ser	Tyr	Ile	Asn	Ile	Ile	Met	Pro	Ser	Val	Phe	Gly
		35					40					45			
Thr	Ile	Cys	Leu	Leu	Gly	Ile	Ile	Gly	Asn	Ser	Thr	Val	Ile	Phe	Ala
	50					55				60					
Val	Val	Lys	Lys	Ser	Lys	Leu	His	Trp	Cys	Asn	Asn	Val	Pro	Asp	Ile
65					70					75				80	
Phe	Ile	Ile	Asn	Leu	Ser	Val	Val	Asp	Leu	Leu	Phe	Leu	Leu	Gly	Met
			85					90						95	
Pro	Phe	Met	Ile	His	Gln	Leu	Met	Gly	Asn	Gly	Val	Trp	His	Phe	Gly
		100						105					110		
Glu	Thr	Met	Cys	Thr	Leu	Ile	Thr	Ala	Met	Asp	Ala	Asn	Ser	Gln	Phe
		115					120					125			
Thr	Ser	Thr	Tyr	Ile	Leu	Thr	Ala	Met	Ala	Ile	Asp	Arg	Tyr	Leu	Ala
	130					135					140				
Thr	Val	His	Pro	Ile	Ser	Ser	Thr	Lys	Phe	Arg	Lys	Pro	Ser	Met	Ala
145					150					155				160	
Thr	Leu	Val	Ile	Cys	Leu	Leu	Trp	Ala	Leu	Ser	Phe	Ile	Ser	Ile	Thr
			165					170						175	
Pro	Val	Trp	Leu	Tyr	Ala	Arg	Leu	Ile	Pro	Phe	Pro	Gly	Gly	Ala	Val
		180						185					190		
Gly	Cys	Gly	Ile	Arg	Leu	Pro	Asn	Pro	Asp	Thr	Asp	Leu	Tyr	Trp	Phe
		195					200					205			
Thr	Leu	Tyr	Gln	Phe	Phe	Leu	Ala	Phe	Ala	Leu	Pro	Phe	Val	Val	Ile
	210					215					220				
Thr	Ala	Ala	Tyr	Val	Lys	Ile	Leu	Gln	Arg	Met	Thr	Ser	Ser	Val	Ala
225					230					235				240	
Pro	Ala	Ser	Gln	Arg	Ser	Ile	Arg	Leu	Arg	Thr	Lys	Arg	Val	Thr	Arg
			245					250						255	
Thr	Ala	Ile	Ala	Ile	Cys	Leu	Val	Phe	Phe	Val	Cys	Trp	Ala	Pro	Tyr
		260						265					270		
Tyr	Val	Leu	Gln	Leu	Thr	Gln	Leu	Ser	Ile	Ser	Arg	Pro	Thr	Leu	Thr
	275						280					285			
Phe	Val	Tyr	Leu	Tyr	Asn	Ala	Ala	Ile	Ser	Leu	Gly	Tyr	Ala	Asn	Ser
	290					295					300				
Cys	Leu	Asn	Pro	Phe	Val	Tyr	Ile	Val	Leu	Cys	Glu	Thr	Phe	Arg	Lys
305					310					315				320	
Arg	Leu	Val	Leu	Ser	Val	Lys	Pro	Ala	Ala	Gln	Gly	Gln	Leu	Arg	Thr
			325					330						335	
Val	Ser	Asn	Ala	Gln	Thr	Ala	Asp	Glu	Glu	Arg	Thr	Glu	Ser	Lys	Gly
		340						345					350		
Thr	Val	Asp	Gly	Thr	Ala	Gly	Pro	Gly	Ser	Ile	Ala	Thr	Met	Val	Ser
		355					360					365			
Lys	Gly	Glu	Glu	Leu	Phe	Thr	Gly	Val	Val	Pro	Ile	Leu	Val	Glu	Leu
	370					375					380				
Asp	Gly	Asp	Val	Asn	Gly	His	Lys	Phe	Ser	Val	Ser	Gly	Glu	Gly	Glu
385					390					395				400	
Gly	Asp	Ala	Thr	Tyr	Gly	Lys	Leu	Thr	Leu	Lys	Phe	Ile	Cys	Thr	Thr
			405					410						415	
Gly	Lys	Leu	Pro	Val	Pro	Trp	Pro	Thr	Leu	Val	Thr	Thr	Leu	Thr	Tyr
		420						425					430		
Gly	Val	Gln	Cys	Phe	Ser	Arg	Tyr	Pro	Asp	His	Met	Lys	Gln	His	Asp
		435					440					445			
Phe	Phe	Lys	Ser	Ala	Met	Pro	Glu	Gly	Tyr	Val	Gln	Glu	Arg	Thr	Ile
	450					455					460				
Phe	Phe	Lys	Asp	Asp	Gly	Asn	Tyr	Lys	Thr	Arg	Ala	Glu	Val	Lys	Phe
465					470					475				480	
Glu	Gly	Asp	Thr	Leu	Val	Asn	Arg	Ile	Glu	Leu	Lys	Gly	Ile	Asp	Phe
				485					490					495	

Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn
 500 505 510
 Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile Lys
 515 520 525
 Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu
 530 535 540
 Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu
 545 550 555 560
 Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp
 565 570 575
 Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala
 580 585 590
 Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys
 595 600

<210> 14
 <211> 673
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> MCH1R-linker-EGFP

<400> 14
 Met Ser Val Gly Ala Met Lys Lys Gly Val Gly Arg Ala Val Gly Leu
 1 5 10 15
 Gly Gly Gly Ser Gly Cys Gln Ala Thr Glu Glu Asp Pro Leu Pro Asn
 20 25 30
 Cys Gly Ala Cys Ala Pro Gly Gln Gly Gly Arg Arg Trp Arg Leu Pro
 35 40 45
 Gln Pro Ala Trp Val Glu Gly Ser Ser Ala Arg Leu Trp Glu Gln Ala
 50 55 60
 Thr Gly Thr Gly Trp Met Asp Leu Glu Ala Ser Leu Leu Pro Thr Gly
 65 70 75 80
 Pro Asn Ala Ser Asn Thr Ser Asp Gly Pro Asp Asn Leu Thr Ser Ala
 85 90 95
 Gly Ser Pro Pro Arg Thr Gly Ser Ile Ser Tyr Ile Asn Ile Ile Met
 100 105 110
 Pro Ser Val Phe Gly Thr Ile Cys Leu Leu Gly Ile Ile Gly Asn Ser
 115 120 125
 Thr Val Ile Phe Ala Val Val Lys Lys Ser Lys Leu His Trp Cys Asn
 130 135 140
 Asn Val Pro Asp Ile Phe Ile Ile Asn Leu Ser Val Val Asp Leu Leu
 145 150 155 160
 Phe Leu Leu Gly Met Pro Phe Met Ile His Gln Leu Met Gly Asn Gly
 165 170 175
 Val Trp His Phe Gly Glu Thr Met Cys Thr Leu Ile Thr Ala Met Asp
 180 185 190
 Ala Asn Ser Gln Phe Thr Ser Thr Tyr Ile Leu Thr Ala Met Ala Ile
 195 200 205
 Asp Arg Tyr Leu Ala Thr Val His Pro Ile Ser Ser Thr Lys Phe Arg
 210 215 220
 Lys Pro Ser Met Ala Thr Leu Val Ile Cys Leu Leu Trp Ala Leu Ser
 225 230 235 240
 Phe Ile Ser Ile Thr Pro Val Trp Leu Tyr Ala Arg Leu Ile Pro Phe
 245 250 255
 Pro Gly Gly Ala Val Gly Cys Gly Ile Arg Leu Pro Asn Pro Asp Thr
 260 265 270
 Asp Leu Tyr Trp Phe Thr Leu Tyr Gln Phe Phe Leu Ala Phe Ala Leu
 275 280 285
 Pro Phe Val Val Ile Thr Ala Tyr Val Lys Ile Leu Gln Arg Met
 290 295 300

Thr Ser Ser Val Ala Pro Ala Ser Gln Arg Ser Ile Arg Leu Arg Thr
 305 310 315 320
 Lys Arg Val Thr Arg Thr Ala Ile Ala Ile Cys Leu Val Phe Phe Val
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 Arg Pro Thr Leu Thr Phe Val Tyr Leu Tyr Asn Ala Ala Ile Ser Leu
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 Glu Thr Phe Arg Lys Arg Leu Val Leu Ser Val Lys Pro Ala Ala Gln
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 Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val
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 Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln
 580 585 590
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 595 600 605
 Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly
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 Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser
 625 630 635 640
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 <212> DNA
 <213> Human

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 <212> DNA
 <213> Mouse

<220>
 <221> misc_feature
 <222> (1)...(2080)
 <223> n = A,T,C or G

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 <212> DNA
 <213> Mouse

<220>
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<211> 1062

<212> DNA

<213> Artificial Sequence

<220>

<223> Human short form/mouse species chimeric MCH1R

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<211> 1269

<212> DNA

<213> Artificial Sequence

<220>

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<211> 966

<212> DNA

<213> Aequorea Victoria

<400> 21

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ctcaaa						966

<210> 22

<211> 765

<212> DNA

<213> Artificial Sequence

<220>

<223> GFP derivative

<400> 22
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 agcgtgtccg gcgagggcga gggcgatgcc acctacggca agctgaccct gaagtccatc 180
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 agcaaagacc ccaacgagaa gcgcgatcac atggtctctg tggagtctgt gaccgcccgc 720
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<210> 23
 <211> 720
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> GFP derivative

<400> 23
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<210> 24
 <211> 720
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> GFP derivative

<400> 24
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<210> 25
 <211> 720
 <212> DNA

<213> Artificial Sequence

<220>

<223> GFP derivative

<400> 25

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<210> 26

<211> 3092

<212> DNA

<213> Artificial Sequence

<220>

<223> Mouse MCH1R-linker-EGFP

<400> 26

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<210> 27

<211> 3056

<212> DNA

<213> Artificial Sequence

<220>

<223> Mouse MCH1R/EGFP direct fusion

<400> 27

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<210> 28

<211> 1815

<212> DNA

<213> Artificial Sequence

<220>

<223> Human short form/mouse species chimeric
MCH1R-linker-EGFP

<400> 28

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<210> 29

<211> 2022

<212> DNA

<213> Artificial Sequence

<220>

<223> Human long form/mouse species chimeric
MCH1R-linker-EGFP

<400> 29

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38

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/08071

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C07K 14/72 , 19/00; C12N 15/62

US CL : 435/69.7, 252.3, 320.1; 530/350; 536/23.4

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.7, 252.3, 320.1; 530/350; 536/23.4

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN, MEDLINE

search terms: fluores?, receptor#, green, g protein, melanin concentrating hormone receptor#

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NELSON et al. Characterization of an Intrinsically Fluorescent Gonadotropin-Releasing Hormone Receptor and Effects of Ligand Binding on Receptor Lateral Diffusion. Endocrinology. February 1999. Vol. 140. No. 2. pages 950-957, see entire document.	1-30
Y	AWAJI et al. Real-Time Optical Monitoring of Ligand-Mediated Internalization of alpha1b-Adrenoreceptor with Green Fluorescent Protein. Molecular Endocrinology. August 1998. Vol. 12. No. 8. pages 1099-1111, see entire document.	1-30

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*G* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

21 JUNE 2001

Date of mailing of the international search report

03 JUL 2001

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/08071

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BACHNER et al. Identification of Melanin Concentrating Hormone (MCH) as the Natural Ligand for the Orphan Somatostatin-Like Receptor 1 (SLC-1). FEBS Letters. 03 September 1999. Vol. 457. No.3. pages 522-524, see entire document.	1-30
Y	SALRO et al. Molecular Characterization of the Melanin-Concentrating-Hormone Receptor. Nature. 15 July 1999. Vol. 400. pages 265-269, see entire document.	1-30